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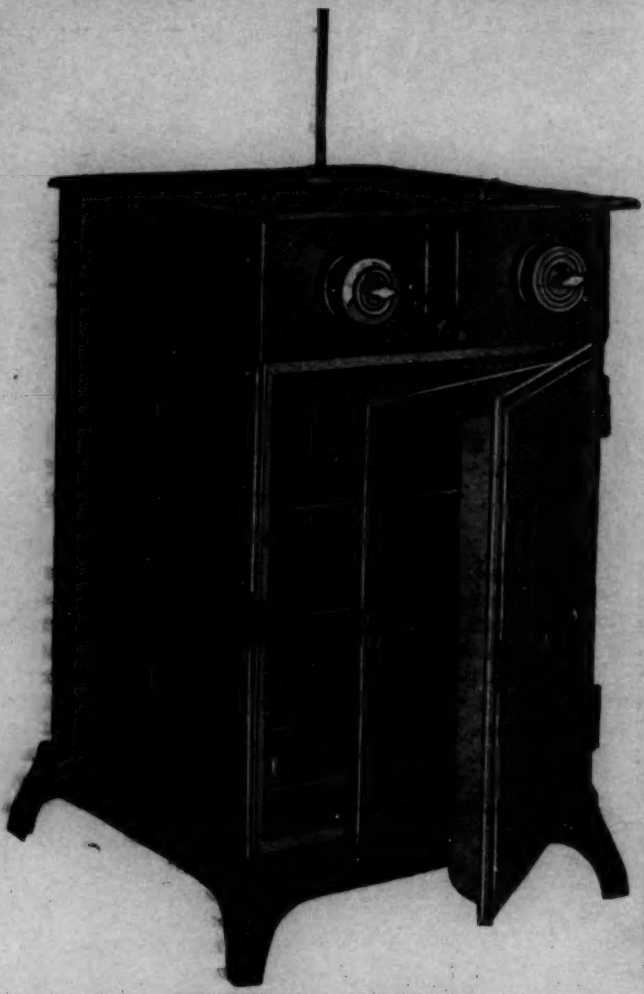

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PROCEEDINGS OF THE AMERICAN SOCIETY OF BIOLOGICAL CHEMISTS.

THIRTEENTH ANNUAL MEETING.

Baltimore, Md., April 24–26, 1919.

DETERMINATION OF AMINO NITROGEN IN COMPOUNDS REACTING SLOWLY WITH NITROUS ACID.

By D. WRIGHT WILSON.

(From the Laboratory of Physiological Chemistry, the Johns Hopkins University, Baltimore.)

The rates of reaction of a number of compounds with nitrous acid were studied. Guanine, guanosine, and guanylic acid each give a quantity of gas equivalent to a considerable fraction more than one nitrogen in 1 to 2 hours at 22–25°. Adenine and adenine nucleotide each give exactly the equivalent of one nitrogen. Xanthine and hypoxanthine give off small quantities of gas but uric acid gives none. Cytosine reacts abnormally yielding 137 per cent of the calculated amount of gas in 3 hours. Uracil, thymine, and uridin do not react.

Allantoin reacts slowly and continuously giving off nearly two nitrogens in 5 hours. Substituted amino groups as found in betaine and sarcosine do not react. While creatine fails to give more than traces of gas in 30 minutes, creatinine reacts rapidly yielding gas equivalent to one nitrogen in 1 hour. The action of nitrous acid on creatinine may account for about one-third of the correction for "slowly reacting amines" met with in Van Slyke's determination of amino nitrogen in the urine. The rate of reaction of the various compounds varies considerably with the temperature.

GROWTH EXPERIMENTS WITH PHASEOLIN FROM THE NAVY BEAN.

By C. O. JOHNS, A. J. FINKS, and MABEL S. PAUL.

(From the Bureau of Chemistry, United States Department of Agriculture, Washington.)

Nutrition experiments with phaseolin, the principal protein of the navy bean (*Phaseolus vulgaris*), show that this protein, after it is treated with dilute alkali and supplemented with 2

per cent of cystine, produces normal growth when it is the sole source of protein in an otherwise adequate diet. Further work is in progress to determine whether alkaline treatment of this protein is necessary for normal growth. The proteins of other beans are being studied in a similar manner.

HYDROLYSIS OF STIZOLOBIN.

By D. BREESE JONES and CARL O. JOHNS.

(*From the Bureau of Chemistry, United States Department of Agriculture, Washington.*)

Stizolobin, the principal protein extracted from the Chinese velvet bean, *Stizolobium niveum*, gave the following percentages of amino-acids on hydrolysis with 20 per cent hydrochloric acid: Glycine 1.66, alanine 2.41, valine 2.88, leucine 9.02, proline 4.00, phenylalanine 3.10, aspartic acid 9.23, serine 0.67, tyrosine 6.24, cystine 1.13, arginine 7.14, histidine 2.27, lysine 8.51, ammonia 1.55. Tryptophane was present. Tyrosine was determined both by the colorimetric method of Folin and Denis, and by direct isolation, the latter method giving 5.25 per cent. A method for the direct determination of proline is described, which consists in removing the bases from the hydrolysis solution by means of phosphotungstic acid, extracting the remaining dry, powdered amino-acids with boiling absolute alcohol. Both the total nitrogen and amino nitrogen in the extract are determined and the percentage of proline is calculated from the resulting data.

DETERMINATIONS OF THE HYDROGEN ION CONCENTRATION OF FOODS DURING STORAGE AND PREPARATION IN RELATION TO PRESERVATION OF ANTISCORBUTIC PROPERTIES.

By J. F. McCLENDON and PAUL F. SHARP.

(*From the Physiological Laboratory, University of Minnesota Medical School, Minneapolis.*)

The juices of green malt, carrots, cabbage, potatoes, turnips, and lean beef were all found to be distinctly acid whether fresh, or after cold storage, or after boiling in an open vessel. The

meat was first triturated in distilled water. The food was simply placed in a canvas bag, the bag placed in a Buchner press, and the juice pressed out. The juice pressed out of boiled food was acid and the juice boiled after pressing out of fresh food was acid. The unboiled preparations became more acid on standing but the boiled preparations were so constant as to indicate that their content of volatile acids or bases was very low.

All measurements were made with the hydrogen electrode in which a gold disc was coated with platinum or palladium black. Results with the disc coated with palladium or only partly immersed could not be duplicated. Results with platinum-coated discs entirely immersed were constant and could be duplicated. Apparently the acids attack palladium and the air-surface film has an abnormal hydrogen ion concentration and must not be near the platinum black coating.

Since antiscorbutic vitamines are sensitive to alkalies the results are encouraging. A malt extract intended to cure scurvy was found to be acid during all steps of preparation. Green malt was ground and mashed and at the end of 1 hour momentarily raised to 70°, and, after all the starch was hydrolyzed, condensed *in vacuo* to the point at which bacteria no longer attacked it.

FAILURE OF ACID-FORMING DIET TO CHANGE THE ALKALINE RESERVE OF DOG'S BLOOD.

By J. F. McCLENDON, OSCAR J. ENGSTRAND, and FRANCES KING.

(*From the Physiological Laboratory, University of Minnesota Medical School, Minneapolis.*)

By titration in the rotating hydrogen electrode it was found that 0.0295 to 0.0300 cc. of normal HCl was required to neutralize 1 cc. of blood plasma on several normal dogs. A dog was fed on raw lean beef for about 2 months. Blood drawn from a vein at the end of 1 month and from the carotid at the end of 2 months and centrifuged without exposure to air was found to have the same alkaline reserve as the controls. The ash of the food would have required 1,500 cc. of normal KOH for neutralization. We believe that acid-forming diets may be detrimental because they may be deficient in vitamines. Cereals and meat (especially as usually cooked) are deficient in antiscorbutic vitamines. A raw meat diet seems to be sufficient for dogs or men.

RELATIVE LENGTH OF THE INTESTINE IS MORE IMPORTANT THAN THE CHARACTER OF THE FOOD IN DETERMINING THE HYDROGEN ION CONCENTRATION OF INTESTINAL CONTENTS.

By J. F. McCLENDON, LEO C. CULLIGAN, CARL S. GYDESEN, and FRANK J. MYERS.

(*From the Physiological Laboratory, University of Minnesota Medical School, Minneapolis.*)

Working on the supposition that we attribute to Metchnikoff that flooding the intestine with soluble carbohydrate in the presence of lactic acid bacteria or *Bacillus acidophilus* should cause an increased hydrogen ion concentration of the ileum, we experimented on pups, dogs, cats, and rabbits. We found the ileum content of pups, dogs, and cats acid throughout the entire length and very little changed by changing the soluble carbohydrate (lactose or other sugar) content of the food. The duodenum and proximal part of the ileum of rabbits are acid but the ileum may become slightly alkaline near the cecum. This alkalinity was not prevented by adding lactose to the diet. On averaging the results of Long and Fenger[1] we conclude that the upper third of the ileum of hogs, calves, and lambs is slightly acid and averages 0.215 atmospheres of CO_2 tension and the lower third is neutral or slightly alkaline and averages 0.14 atmospheres of CO_2 pressure. Apparently the CO_2 generated by the action of the gastric contents on the pancreatic juice makes the reaction acid and this acidity is kept up in a short intestine by amino-acids. In a long intestine the CO_2 and amino-acids are largely absorbed before the cecum is reached and the reaction may become alkaline. If acid fermentation of sugars occurs the acids are absorbed so rapidly that they do not prevent the alkaline reaction in the long intestine of herbivora. By forced feeding with sugar a marked diarrhea may be produced and a slightly increased acidity result. The replacement of *Bacillus acidophilus* by *Bacillus coli* in the weening of pups does not result in a marked lowering of the hydrogen ion concentration of the ileum.

[1] Long, J. H., and Fenger, F., *J. Am. Chem. Soc.*, 1917, xxxix, 1278.

FAT-SOLUBLE VITAMINE OF GREEN FOODS.

By THOMAS B. OSBORNE and LAFAYETTE B. MENDEL.

(From the Laboratory of the Connecticut Agricultural Experiment Station and the Sheffield Laboratory of Physiological Chemistry, Yale University, New Haven.)

Our evidence for the occurrence of fat-soluble vitamine in certain green foods,[2] which has hitherto been demonstrated only by the use of the food products as a whole in the diet can now be supplemented with experiments in which ether extracts of the plant tissues have proved similarly efficient. McCollum, Simmonds, and Pitz[3] have stated that "ether extraction of plant tissues does not remove the substances essential for growth which is contained in butter fat." We have, however, obtained potent preparations as follows: Spinach leaves and young clover respectively, dried in a current of air at about 60°, were extracted with U. S. P. ether. The resultant green extract, yielding an oily residue equal to about 3 per cent of the dried plant, was evaporated upon starch. These preparations, fed in daily quantities equivalent to 1 to 2 gm. of the dried plant, promoted recovery and renewal of growth in rats declining on diets deficient in fat-soluble vitamine. Inasmuch as only 30 mg. per day of the ether extract of spinach sufficed for this purpose it appears that this product ranks among the most potent of the oils heretofore tested. The effects of spinach oil and clover oil in restoring growth in rats that had declined on a diet lacking the fat-soluble vitamine were exhibited in graphic charts of changes in body weight.

ARE THE ANTINEURITIC AND THE WATER-SOLUBLE B VITAMINES THE SAME?

By A. D. EMMETT and G. O. LUROS.

(From the Research Laboratory of Parke, Davis and Company, Detroit.)

The chief point in this series of experiments was to study the effect of feeding the same basal food, natural brown rice, to *both* pigeons and young rats, and to determine the effect on the onset

[2] Osborne, T. B., and Mendel. L. B., *J. Biol. Chem.*, 1919, xxxvii, 187.

[3] McCollum, E. V., Simmonds, N., and Pitz, W., *Am. J. Physiol.*, 1916, xli, 363.

of polyneuritis and on the rate of growth. The rice was the sole source of the water-soluble vitamine. It was supplemented, for the rats, so that the diet was complete in all other respects. The effect of heat on the water-soluble vitamine in the rice was the criterion for making the distinction.

It was found, upon heating the rice for 2 and 6 hours respectively in the autoclave at 15 pounds pressure, that the anti-neutritic vitamine was destroyed while the growth-promoting vitamine (water-soluble B) was not so altered. The rate of growth and the food intake varied inversely with the length of time of heating.

We took vitamine extracts of brewer's yeast, and activated fullers' earth vitamine preparations from yeast and protein-free milk, heated these in the same manner as the rice, and tried them out on rats and pigeons. Pigeons could not be cured of polyneuritis nor could the onset of the disease be delayed. Young rats that were suffering from the lack of the water-soluble B immediately began to grow. The possibility of toxic substances in the heated rice having thus been removed, the evidence seemed to be clear that these two vitamines were not one and the same as has been generally claimed. Further study is in progress to enable us to substantiate this hypothesis.

STABILITY OF LACTALBUMIN TOWARDS HEAT.

By A. D. EMMETT and G. O. LUROS.

(*From the Research Laboratory of Parke, Davis and Company, Detroit.*)

Since casein has been claimed to be susceptible to heat and also to be a better protein for growth than lactalbumin, the nutritive value of the latter was studied from the standpoint of its stability toward high temperature. Lactalbumin was heated in an air oven for 2 hours at 120°, and in an autoclave for 1, 2, and 6 hours respectively at 15 pounds pressure, temperature 120°. The heated protein was incorporated in a ration which was complete for normal growth in all respects. Control tests were carried out with lactalbumin that had been dried *in vacuo* at 55–60°. The amount of fat-soluble A (butter fat) was varied also ranging from 5 to 28 per cent.

It was found that heating had practically no effect upon the nutritive value of the lactalbumin even when heated for 6 hours in the autoclave. The higher amounts of butter fat rendered the rations more economical, that is, of the three groups of rats on the 2 hour autoclaved lactalbumin, fed respectively 5, 18, and 28 per cent fat, the 28 per cent fat diet made better gains per gm. of food than did the 5 per cent fat diet. The effect of heat appears to be due to the partial destruction of a water-soluble vitamine other than the growth-promoting accessory, water-soluble B.

NUTRITIONAL STUDIES ON GROWTH OF FROG LARVÆ (RANA PIPIENS).

By A. D. EMMETT and FLOYD P. ALLEN.

(*From the Research Laboratory of Parke, Davis and Company, Detroit.*)

Tadpoles soon after hatching were grouped and each lot of 500 was fed a definite diet which varied in respect to the kind and amount of protein, the kind and source of water-soluble and fat-soluble vitamines, the kind of carbohydrate, and the amount of fat. Detailed observations were made of the rate of growth (size) and development (length of hind legs). Experimental conditions were maintained exactly the same for all groups including the controls.

It was found that the quality of protein was a much more important factor than the amount; that both vitamines were essential, the water-soluble type being perhaps the more important; that dextrin was no more available than starch; and that the amount of fat present was a very important factor to be borne in mind—large amounts inhibiting both growth and development. In these preliminary studies, it was evident that some factor or factors other than those which were needed for normal growth and development of the rat was essential to the full, vigorous maturity of tadpoles.

DISTRIBUTION OF THE ANTINEURITIC VITAMINE IN THE WHEAT AND CORN KERNEL.

By CARL VOEGTLIN and C. N. MYERS.

(*From the Division of Pharmacology, Hygienic Laboratory, Washington.*)

Feeding experiments on pigeons with corn and wheat, from which the portion containing the embryo had been eliminated, have shown that these foods are practically devoid of antineuritic vitamine. The pigeons developed polyneuritic symptoms after 3 to 4 weeks. Alcoholic extracts of the portion containing the germ, when administered to these polyneuritic pigeons, relieved the symptoms. When changed to an exclusive diet of whole wheat or corn the pigeons also recovered and remained healthy for weeks. It is therefore concluded that (1) the aleurone cells are not the seat of the antineuritic vitamine and (2) that this substance resides in the portion containing the germ and probably within the germ.

COMPARATIVE METABOLISM OF P-NITROPHENYLACETIC ACID.

By CARL. P. SHERWIN.

(*From the Chemical Laboratory of Fordham University Medical School, New York.*)

After the ingestion of *p*-nitrophenylacetic acid by a man, only the free acid could be found in the urine and none apparently existed in a conjugated form. 68.70 per cent of the acid fed was extracted from the urine.

After feeding the acid to a dog only 45 per cent of the amount fed was recovered from the urine. Of this amount, 30 per cent was excreted as free *p*-nitrophenylacetic acid while 15 per cent was combined with glycocoll and excreted as *p*-nitrophenaceturic acid.

A hen was fed small doses (1 gm. or less) of the *p*-nitrophenylacetic acid. Some of the free acid was extracted from the excreta, but most of the acid was found to be combined with ornithine. This compound on analysis gave results corresponding to the formula $C_{21}H_{22}N_4O_8$.

The acid is soluble in alcohol but insoluble in ether and cold water. The sodium and potassium salts were found to be very hygroscopic and in water solution were dextrorotatory.

The compound was hydrolyzed by boiling with 30 per cent HCl. *p*-Nitrophenylacetic acid was extracted with ether from this acid mixture and identified. The HCl solution remaining was evaporated *in vacuo*. The residue was made alkaline and shaken with benzoyl chloride, then acidified. Crystals of ornithinic acid appeared.

The compound isolated from the excreta of the hen is undoubtedly *p*-nitrophenacetornithinic acid, composed of 1 molecule of ornithine and 2 molecules of *p*-nitrophenylacetic acid.

NATURE OF THE TOXIC AGENT IN MEAT POISONING.

By ISIDOR GREENWALD.

(From the Harriman Research Laboratory, the Roosevelt Hospital, New York, in Cooperation with the Section of Food and Nutrition, Medical Department, United States Army.)

Fresh beef was infected with cultures of *Bacillus paratyphosus* B, *Bacillus enteriditis*, etc. After standing for from 2 to 10 days, the organisms were killed by heating the jar containing the meat to 80° for 30 minutes. The meat was then fed, for from 6 to 8 days, to rats, mice, and guinea pigs. No ill effects were observed. Similar results were obtained with beef and veal condemned because of septicemia, etc. in the animal.

Precipitation by mercuric acetate cannot be employed for the isolation of methylguanidine. The precipitation is not complete and methylguanidine is formed by the oxidation of creatine. Using a method which is free from these objections, no methylguanidine could be isolated from either fresh meat or that which had been allowed to undergo several days bacterial decomposition. Added methylguanidine was recovered, quantitatively.

PRELIMINARY EXPERIMENTS OF THE INFLUENCE OF AMINO-ACID ON THE DIASTATIC HYDROLYSIS OF STARCH.

By H. C. SHERMAN and FLORENCE WALKER.

(From Columbia University, New York, and Carnegie Institution of Washington, Washington.)

Wheat, maize, and rice starches, similarly purified, showed equal digestibility or rate of diastatic hydrolysis. This was true whether the enzyme employed was purified pancreatic amylase,

commercial pancreatin, saliva, purified malt amylase, malt extract, purified amylase of *Aspergillus oryzæ*, or commercial taka-diastase. With all these except purified pancreatic amylase, potato starch showed a rate of hydrolysis equal to or slightly greater than that observed with the cereal starches. The tendency to abnormally low results in the case noted can be corrected by the addition either of a boiled, carefully neutralized, water extract of potato, or neutralized aspartic acid. Neutralized asparatic acid also increased the rate of transformation of all four of the starches tested, when the enzyme employed was purified pancreatic or malt amylase, commercial pancreatin, or saliva, but did not influence the rate of action of malt extract or taka-diastase. It was demonstrated electrometrically that the neutralized amino-acid added in these experiments was without effect upon the hydrogen ion concentration of the digestion mixture. The experiments are being continued and extended to other amino-acids and acid amides.

A CORRELATION ON THE OCCURRENCE OF THE FAT-SOLUBLE VITAMINE.

By H. STEENBOCK, P. W. BOUTWELL, and HAZEL E. KENT.

(*From the Laboratory of Agricultural Chemistry, University of Wisconsin, Madison.*)

In the generalizations sometimes indulged in, when evaluating naturally occurring foodstuffs for specific dietary properties, there has been a tendency to associate a high concentration of the vitamines with the metabolically more active tissues. While this may have been justifiable in certain instances, yet such teleological reasoning may lead to conclusions far from the truth. This has been brought out in experiments carried out to determine the fat-soluble vitamine content of tubers and roots. Presumably all tubers and roots function in the capacity of storage organs for the storage of materials necessary for nursing the growth of the following year's sprouts, yet among them there is a tremendous variation in the fat-soluble vitamine content. This is of such an order that it cannot be correlated with difference in amount of growing tissues. However, there appears to be some relation between yellow pigmentation and vitamine content.

Sweet potatoes and carrots, both highly pigmented, are very rich in the fat-soluble vitamine, while Irish potatoes, mangels, dasheens, and sugar beets, all carrying little or no pigment, are poor in it. Somewhat similar relations obtain with maize, although it is not as yet justifiable to state it as a general principle. On sound kernels of white maize, suitably supplemented with protein and salts, it has been found impossible to keep young rats alive longer than a few months—death usually resulting after xerophthalmia had set in. On the other hand, on yellow maize, under the same experimental conditions, young rats have grown to maturity at the normal rate, have maintained themselves in excellent condition for months, and have reproduced repeatedly. It is certain that maize, as one of our cereal grains, is by no means always as deficient in the fat-soluble vitamine as present day statements in current contributions would lead one to believe. It is possible that these observations of pigment and vitamine content may give a clue to the manipulations necessary in a procedure leading to the isolation of the fat-soluble vitamine. It is not meant to infer that the vitamine is necessarily a colored compound, but its association with yellow plant pigments is suggestive of its possible chemical nature. It is not carotin.

ON THE PROBLEM OF THE PRODUCTION OF FAT FROM PROTEIN IN THE DOG.

By H. V. ATKINSON and GRAHAM LUSK.

(*From the Physiological Laboratory of Cornell University Medical College, New York.*)

In eight experiments in a series of thirteen, after giving meat in large quantities (700 to 1,300 gm.) to a dog weighing 11 kilos, the respiratory quotients during the height of protein metabolism were between 0.793 and 0.800. A calculation showed that under these conditions the retained carbon residue of the protein metabolized was held back in such a form that, had it been oxidized, the respiratory quotient of this retained pabulum would have been 0.85. This would represent the oxidation of material half of whose calories were composed of fat and half of carbohydrate. The dog showed quotients of 0.82 and above only after the larger quantities of meat were given (1,000 gm. or more). It was

extremely difficult to induce the dog to take meat in sufficient quantity to indicate a considerable production of fat from protein. Incidentally it was observed that the basal metabolism of a dog fed with meat in large quantity for a time and then caused to revert to a standard diet (meat, 100 gm.; lard, 20 gm.; and biscuit meal, 100 gm.) remained persistently (even after 2.5 weeks) at a higher level than had obtained prior to the meat ingestion. This confirms F. G. Benedict's idea of a higher basal metabolism in the presence of "surplus" cellular nitrogen, or the "improvement quota" of protein according to Rubner's terminology.

NOTE ON THE COLORIMETRIC DETERMINATION OF LACTOSE.

By JOSEPH BOCK.

(*From the Department of Physiological Chemistry, Marquette School of Medicine, Milwaukee.*)

The reaction of lactose with picric acid was studied. The procedure used is similar to the one used by S. R. Benedict in the colorimetric determination of dextrose. The lactose is hydrolyzed before being heated with picric acid and sodium carbonate. The unhydrolyzed lactose gives less uniform results and less intense color development.

The lactose in milk is being determined by this method. After hydrolyzing the lactose, the milk proteins are removed by precipitation with picric acid. An aliquot of the filtrate is used for the lactose determination. 1 cc. or less of milk is used.

Details of the procedure will be published in the near future. The reaction of other carbohydrates with picric acid is being studied.

URINARY OUTPUT OF NITROGEN, CHLORINE, CALCIUM, AND MAGNESIUM IN DIABETES MELLITUS.

By C. FERDINAND NELSON.

(*From the Laboratory of Biological Chemistry, University of Kansas, Lawrence.*)

Determinations of calcium, magnesium, and sodium chloride in nineteen cases of diabetes mellitus show variations as follows:

Calcium (metal)	100– 791 mg.
Magnesium "	88–1,049 "
Sodium chloride	1.7–15.83 gm.

The total nitrogen varied from 4.58 to 18.40 gm.

Magnesium was excreted in amounts larger than calcium in 26 per cent of the cases studied.

Eleven determinations, made from 5 to 21 days after six of the above cases had been rendered free from sugar, showed a striking change in the urinary calcium and magnesium ratio, magnesium appearing in larger amounts than calcium in 72 per cent of the determinations.

The total nitrogen in each of the latter cases was from 1 to 6 gm. higher than in the former.

CALCIUM AND MAGNESIUM CONTENT OF NORMAL URINE. II.

By C. FERDINAND NELSON.

(From the Laboratory of Biological Chemistry, University of Kansas, Lawrence.)

Additional determinations of the urinary output of calcium and magnesium in normal men and women, on mixed diets in no way restricted or modified except by appetite, show in 58 cases (41 men and 17 women) extreme variations of calcium and magnesium as follows:

			mg.
Calcium	(metal)	Men	416–87
		Women	265–28
Magnesium	"	Men	305–30
		Women	166–39

Calcium occurs in larger amounts than magnesium in urine in from 74 to 84 per cent of all determinations made in this laboratory. Magnesium predominates in from 16 to 24 per cent.

Analyses of 3 hour samples of urine, in cases where the magnesium output exceeds that of calcium, show a constant preponderance of the former element throughout the periods observed.

The nitrogen output in cases where magnesium exceeds calcium in the urine is quite as high or may be even higher than where calcium is excreted in the larger amount.

The ingestion of calcium-rich foods, such as milk, promptly increases both the urinary calcium and magnesium outputs.

AVERAGE FOOD CONSUMPTION IN TRAINING CAMPS OF THE UNITED STATES ARMY.

By JOHN R. MURLIN,

Lieutenant Colonel, Sanitary Corps, U. S. Army.

(From the Division of Food and Nutrition, Medical Department, United States Army.)

A comparison was shown of the various rations of the Allied armies, those used in the training period, and those used in active campaign. The average food consumption in 427 messes scattered over 67 different camps including forty-nine divisional and other large concentration camps, fourteen aviation fields, three war prison barracks, one recruiting station, and one spruce production camp is 3,633 calories. The weighted average amounts to 3,625 calories. To this must be added the average food consumption from the canteen or post exchange. Studies in 261 post exchanges in these camps disclose an actual average *per capita* consumption of 365 calories daily. This makes a total food consumption for the average soldier in training of in round numbers 4,000 calories (3,998). Averages by months indicate a well marked, though slight, seasonal variation. The same is shown also when the various messes studied are arranged according to the average prevailing temperature at the time of the surveys.

As compared with the consumption in civilian households the army uses more than twice as much fresh meat, beans, and dried fruit, one and a half times as much bread and bakery products and potatoes, the same amount of sugar, eggs, and lard, much less fresh vegetables, fresh fruits, butter, and milk.

Of the 4,000 calories consumed by the average soldier in heavy training 13 per cent is protein, 31 per cent fat, and 56 per cent carbohydrate. Neglecting the canteen consumption, the distribution is 14 per cent protein, 31 per cent fat, and 55 per cent carbohydrate. As compared with civilian dietaries the percentage of protein is higher, the percentage of fat about the same, and the percentage of carbohydrate a little less.

The average recruit on this diet has gained nearly 7 pounds in a period of 5 months training; the gain is evenly distributed over men of different initial weight.

VARIATIONS IN STRENGTH AND IN THE CONSUMPTION OF FOOD BY RECRUITS AND BY SEASONED TROOPS.

By PAUL E. HOWE,

Captain, Sanitary Corps, U. S. Army.

(From the Section of Food and Nutrition, Medical Department, United States Army.)

Determinations were made of the variations in strength, using the Martin strength test (by Lieut. C. C. Mason), and weight of approximately forty men from each of eight companies of recruits. The studies extended for 3 consecutive weeks; a smaller number of men were studied for 4 weeks. Weights and strength were determined at 7 day intervals. The food consumption of the companies from which the men were taken was determined (by Lieut. S. C. Dinsmore) in weekly periods. In each period one group of four companies used the same menu and the other four companies a different menu but applicable to all four companies. The men were inoculated and vaccinated during the first 2 weeks of study.

In the first test the men had an average strength which was rather high grade for civilians according to Martin's classification (low B), 757 lbs. Both strength and weight had decreased on the average at the second test: weight, 145.7 to 144.7 lbs., strength, to 705 lbs. The third test indicated that the men had returned approximately to normal: weight, 146.3 lbs.; strength, 743 lbs. The men tested the fourth time maintained their status of the 3rd week or increased slightly in both weight and strength. Conflicting values were obtained with regard to the relation of the proximity of the inoculation to the succeeding strength test. The changes in weight agree with those obtained by Lieut. Perlzweig in another camp.

The average consumption of food for all the companies shows a decrease in the 2nd week from 3,436 to 3,248 calories per man per day; the following week there was an increase to 3,542 calories. One of the two groups of four companies showed a gradual decrease in food consumption while the other showed a regular increase. The food ingestion of different groups of men using the same menu showed considerable variations in quantity and the same group of men have a tendency to vary in the quantity of

food consumed on the basis of the weekly average consumption. These variations are apparently not related to weather changes. The variation in food consumption of seasoned troops, four companies, over a period of 4 weeks likewise showed considerable variation. In each comparative case the men were doing approximately the same work.

EFFECT OF INTRAVENOUS INJECTION OF PANCREAS EMULSION UPON THE HYPERGLYCEMIA DUE TO DEPANCREATIZATION.

By ISRAEL S. KLEINER.

(*From the Department of Physiology and Pharmacology of The Rockefeller Institute for Medical Research.*)

4 years ago, in the course of another investigation, Kleiner and Meltzer[4] found that an intravenous infusion of pancreas emulsion into depancreatized dogs temporarily reduced the blood sugar in a marked degree—sometimes to a normal level. The three experiments of that report have now been increased to sixteen, with results which confirm the preliminary statement.

The pancreatic emulsion was prepared as follows: Fresh dog's pancreas was hashed and mixed with three or four times its weight of sterile distilled water. After from 1 to 20 hours in the refrigerator it was strained and squeezed through muslin. The fluid was then mixed with five volumes of sterile 0.9 per cent NaCl solution and was injected slowly during the course of about an hour. The injection of this unfiltered faintly acid or neutral fluid caused a marked reduction of the sugar content of the blood (a loss of 0.09 to 0.20 per cent) in ten experiments, while in six the fall was less pronounced (0.02 to 0.07 per cent). For example, in two experiments the glycemia fell from 0.28 to 0.08 per cent and from 0.33 to 0.17 per cent, respectively. The fall began during the injection and reached its maximum 1 or 2 hours after the injection was ended. There was no dilution of the blood.

A reduction in glycosuria also occurred, but this was produced also by emulsions of other organs. These, however, have not produced the above effect on the blood sugar.

[4] Kleiner, I. S., and Meltzer, S. J., *Proc. Nat. Acad. Sc.*, 1915, i, 338.

OXIDATION OF LUCIFERIN AND REDUCTION OF OXYLUCIFERIN OF LUMINOUS ANIMALS.

By E. NEWTON HARVEY.

(From the Physiological Laboratory, Princeton University, Princeton.)

Some luminous animals are known to produce their light by the oxidation of a substance called luciferin in the presence of a second substance called luciferase. Luciferase has many characteristics of an enzyme but is slowly used up in oxidizing large quantities of luciferin. A third substance, photophelein, may assist the luciferin-luciferase reaction. In the absence of luciferase, luciferin oxidizes spontaneously but without light production. The oxidation product may be called oxyluciferin. The reaction luciferin⇄oxyluciferin is similar to the reaction leucomethylene blue⇄methylene blue, as the oxyluciferin can be reduced to luciferin again by Schardinger's enzyme of milk or the reductases of animal tissues. Oxyluciferin can also be reduced by H_2S, nascent hydrogen (from Mg powder and acid), by finely divided palladium, and sodium hypophosphite. Dilute acid favors the reduction and dilute alkali favors the oxidation change. Contrary to the opinion of most investigators no extensive oxidative change appears to occur during luminescence and no carbon dioxide is formed.

ENZYME STUDIES ON DEHYDRATED AS COMPARED WITH FRESH VEGETABLES.

By K. GEORGE FALK, GRACE McGUIRE, and EUGENIA BLOUNT.

(From the Harriman Research Laboratory, the Roosevelt Hospital, New York, in Cooperation with the Section of Food and Nutrition, Medical Department, United States Army.)

Oxidase, peroxidase, catalase, and amylase were determined in fresh and dehydrated potatoes, tomatoes, cabbage, yellow and white turnips, and carrots. In general, the enzyme actions were decreased on dehydration, more so by air blast dehydration than by vacuum dehydration. The effects of hydrogen ion concentrations on the actions were studied. The potato amylase apparently acted differently on the starch occurring naturally in potato juice than it did on Lintner prepared starch.

THE WORK OF THE HARRIMAN RESEARCH LABORATORY IN AFFILIATION WITH THE DIVISION OF FOOD AND NUTRITION, MEDICAL DEPARTMENT, UNITED STATES ARMY.

By K. GEORGE FALK.

(From the Harriman Research Laboratory, the Roosevelt Hospital, New York, in Cooperation with the Division of Food and Nutrition, Medical Department, United States Army.)

The work was divided into three parts: study of meat, including chemical study of spoilage[5] and toxicity studies; development of new process of dehydration applicable to all food products including meat and fish; comparative study of enzymes, proteins, and carbohydrates of fresh vegetables and vegetables dehydrated by different processes.

CHEMICAL CHANGES IN THE BLOOD IN ADVANCED NEPHRITIS.

By VICTOR C. MYERS and JOHN A. KILLIAN.

(From the Laboratory of Pathological Chemistry, New York Post-Graduate Medical School and Hospital, New York.)

As is now well known, the blood in cases of advanced (interstitial) nephritis is characterized by a marked increase in all the compounds which go to make up the non-protein nitrogen. Of the three waste products, uric acid, urea, and creatinine, creatinine appears to be most readily eliminated by the kidney, and is therefore the last one to be retained in nephritis. For this reason and also because of its endogenous origin and very constant formation, the kidney is apparently never able to overcome the handicap of a high creatinine accumulation, thus making creatinine a most valuable prognostic test. Of 85 cases having a creatinine of over 5 mg. per 100 cc. of blood (figures up to 33 mg.) 80 have died, three remain unchanged, while two acute cases have recovered (figures of 5.6 and 6.1 mg.). The question naturally arises whether creatinine may not be responsible for some of the terminal symptoms as a result of its conversion to methylguanidine, a point which has not yet been definitely answered.

[5] Falk, K. G., Baumann, E. J., and McGuire, G., *J. Biol. Chem.*, 1919, xxxvii, 525. Falk, K. G., and McGuire, G., *J. Biol. Chem.*, 1919, xxxvii, 547.

In connection with convulsive symptoms it may be noted that low figures for Ca may be found; we have observed figures as low as 3 to 4 mg. per 100 cc. of blood.

Judging from the first twenty cases in the above series, in which CO_2 estimations were made, severe acidosis is an invariable accompaniment of advanced nephritis, the CO_2 in twelve instances being low enough to afford an explanation of coma and death.

ELIMINATION OF TARTRATES.

By GEORGE ERIC SIMPSON.

(From the Department of Experimental Medicine, School of Medicine, Yale University, New Haven.)

The urinary elimination of dextro tartaric acid was studied after the sodium salt was given subcutaneously or by stomach tube to cats, dogs, and rabbits. The method used for the determination of tartrate was the Halenke and Moeslinger procedure, which involves the titration of precipitated potassium acid tartrate with standard alkali. This method was found to give consistent results when applied to urine.

After subcutaneous administration, tartrate is eliminated in the urine of all species studied in practically the same degree. Between 70 and 90 per cent of tartrate was found in the urine. When successive subcutaneous injections were given without the development of tolerance the amount of tartrate excreted after each injection decreased until finally none was excreted and the animals died.

When tartrate was given to rabbits or dogs by mouth less was eliminated by the kidneys than after subcutaneous administration. In rabbits an average of 4.9 per cent, in dogs an average of 23.9 per cent, of the tartrate administered by stomach tube was found in the urine. It seems hardly possible that tartrates are oxidized within the body. That part which passes the intestinal wall is for the most part excreted unchanged in the urine.

Calcium administration was not found to delay the excretion of tartrate or alter the degree of elimination by the kidneys. Thus calcium precipitation is probably not a factor in tartrate nephritis.

We were unable to recognize any marked effect of diet on the urinary excretion of tartrates, or to explain, by our study, the marked effect of diet on toxicity reported by Salant and his coworkers.

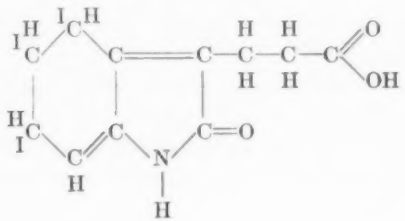
CHEMICAL IDENTIFICATION OF THE THYROID HORMONE.

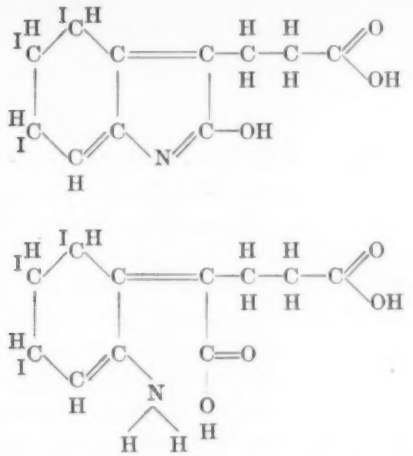
By E. C. KENDALL.

(*From the Section of Biochemistry, Mayo Foundation, Rochester.*)

The iodine-containing compound which occurs in the thyroid was isolated in crystalline form at the end of the year 1914. Its empirical and structural formulas were determined in May, 1917, and now its synthesis, which has recently been accomplished, completes the chemical work involved in this present investigation. Its chemical structure is related to that of tryptophane, from which it is probably derived. The compound has been named thyro-oxy-indol which has been abbreviated to thyroxin. The data concerning its formula and derivatives were given and the active groups in the molecule CO-NH were discussed. The iodine in the molecule which is attached to the benzine ring does not appear to be involved when the substance affects the energy output, but the chemical groups responsible for its activity are the CO-NH groups which in the body change their form to amino carboxyl groups and the substance, in all probability, functions in this form. The change from CO-NH to amino carboxyl is precisely similar to the opening of the creatinine ring with the formation of creatine. Thyroxin, therefore, although not an α-amino-acid, is essentially an amino-acid and it falls into the well known fundamental groups of substances: amino-acids, protein, creatinine, creatine, etc.

Thyroxin can exist in three forms. The structural formulas are as follows:





DETERMINATION OF ACETONE IN EXPIRED AIR.

By ROGER S. HUBBARD.

(From the Laboratory of Clifton Springs Sanitarium, Clifton Springs.)

A method was described for determining quantitatively acetone in the expired air. Acetone was collected in dilute solution of sodium bisulfite. The subject breathed directly through two bottles containing a 2.5 per cent solution. It was transferred to a Kjeldahl flask and distilled from sulfuric acid and excess potassium permanganate. It was then redistilled from sodium peroxide into Scott-Wilson reagent, and determined by comparing the turbidity with that produced by known amounts of acetone freshly distilled into the same reagent. Solutions of pure acetone added to the breath or to a stream of carbon dioxide gave 90 to 100 per cent recovery.

Normal excretion of acetone per hour as determined by this method ranged from 0.12 to 0.60 mg. and was considerably affected by the diet. Cases showing acidosis gave considerably higher values.

DIRECT DETERMINATION OF THE NON-AMINO NITROGEN IN HYDROLYZED PROTEINS.

By ALMA HILLER.

(*From the Laboratories of The Rockefeller Institute for Medical Research.*)

The former method used by Van Slyke in determining the non-amino nitrogen in hydrolyzed proteins was an indirect one, calculated by subtracting the amino nitrogen from the total nitrogen of the amino-acids. The present method determines this nitrogen directly by employing the following four steps:

1. The removal of phosphotungstic acid from the solution by means of an amyl alcohol-ether mixture, shaking in a separatory funnel, which completely removes the phosphotungstic acid.

2. The removal of the amino nitrogen by means of nitrous acid, in the presence of 10 per cent hydrochloric acid, and by the aid of heat.

3. The removal of the nitrous acid introduced into the reaction by means of reduction with zinc-copper couple.

4. The determination of the remaining or non-amino nitrogen by means of the Kjeldahl method.

INFLUENCE OF TEMPERATURE ON THE ANTISCORBUTIC VITAMINE IN TOMATOES.

By MAURICE H. GIVENS and HARRY B. McCLUGGAGE.

(*From the Department of Physiology, University of Rochester, Rochester.*)

Experiments have been conducted to determine the effect of heat upon the value of tomatoes as an antiscorbutic agent. A daily supplement of 2.5 gm. of fresh raw tomatoes will protect a guinea pig from experimental scurvy when that animal is fed a scurvy-producing diet which is otherwise adequate. If fresh tomatoes are heated at 100°C. for 15 minutes or longer a larger daily dosage must be supplied to insure the animal protection against the disease.

Tomatoes canned according to the usual method of processing, heating under 5 pounds pressure for 10 minutes, will prevent scurvy in a guinea pig when fed in daily portions of 10 gm. This quantity is also sufficient for protection even after heating at 15 pounds pressure for 30 minutes.

Tomatoes dried at 35–40°C. for 32 to 52 hours will protect a guinea pig against experimental scurvy when fed in a daily amount of 0.5 gm. However, this dosage must be increased if the dried tomatoes are cooked before feeding.

The results obtained indicate that the tomato is a very efficient antiscorbutic agent. Further, there is some reduction in its antiscorbutic potency if it is subjected to certain temperature treatments.

THE ISOELECTRIC POINT OF VEGETABLE PROTEIN.

By E. J. COHN.

(*From the Harriman Research Laboratory, the Roosevelt Hospital, New York, in Cooperation with the Division of Food and Nutrition, Medical Department, United States Army.*)

The chief protein constituent of the potato—tuberin—was studied in connection with an investigation conducted by the Division of Food and Nutrition of the Surgeon General's Office upon the nature of the proteins in certain vegetables. The isoelectric point of tuberin was determined by the method of cataphoresis, and found to coincide with a slightly smaller hydrogen ion concentration than 10^{-4} N. At less acid and at neutral reactions, the protein ionized as anion and migrated to the anode in an electric field. Only at hydrogen ion concentrations greater than 10^{-4} N did the direction of migration change and tuberin ionize as cation.

The effect of sodium chloride upon the solubility of tuberin at different hydrogen ion concentrations illustrates the significance of the isoelectric point. Only at the isoelectric point has sodium chloride no effect upon the solubility of tuberin. At greater acidities, the solubility was decreased by an increase in the concentration of sodium chloride. At neutral reactions sodium chloride increased the solubility of tuberin, which has therefore always been classified as a globulin.

The addition of sodium chloride also has an effect upon the apparent hydrogen potential of otherwise identical systems containing either tuberin and an acid or tuberin and a base. The addition of sodium chloride caused the reaction to converge upon a definite acidity. The addition of salt allows protein to combine

with more acid or more alkali at the same pH everywhere but at the isoelectric point. Previously published data upon gluten claim that gluten exhibits this phenomenon as did tuberin prepared by precipitation at the isoelectric point. Tuberin prepared by dialysis differed in its behavior in that the acidity of systems containing it occasionally converged upon another reaction, characteristic of the method of preparation. Under these circumstances, changes in solubility of the tuberin preparation accompanied those in reaction. These methods of investigation have also been employed in the study of the carrot and the tomato.

COMPOSITION OF BOG BUTTER COMPARED WITH THAT OF ADIPOCERE.

By R. F. RUTTAN and L. ISOBEL HOWE.

(From the Department of Chemistry, McGill University, Montreal.)

Eight specimens of bog butter obtained from the Royal Irish Academy of Dublin and seven samples from the Belfast Public Art Museum were analyzed. In Table I will be found the physical and chemical constants of these specimens as compared with those of a very mature sample of pig's adipocere.

The analysis showed:

1. That the hydrolysis of the fats is not so complete in the bog butter as in the adipocere and the percentage of unsaturated fat acids is larger in the bog butter.

2. That the two hydroxy stearic acids which were found by the authors to be present in all samples of adipocere were also found in every sample of the fifteen examined, the average proportion being similar to that found in mature adipocere.

The specimens of bog butter will be seen to vary greatly among themselves in their general composition but like adipocere they have a higher melting point than the original fat and consist essentially of palmitic acid mixed with the two hydroxy stearic acids and a variable but small quantity of oleic acid and fats. Two specimens gave traces of volatile fat acids. Unlike adipocere, soaps were usually absent; traces only were found in one or two specimens.

The theta- and iota-hydroxy stearic acids may be considered characteristic of all "fossil fats."

TABLE I.

Physical and Chemical Constants of Bog Butter Compared with Those of Mature Pig's Adipocere.

	Mature pig's adipocere.	Bog butter.		
		Minimum.	Maximum.	Average of 15 specimens.
Specific gravity (100°C.)	0.8436			0.8432
Soluble in ether	94.1	94.2	98.85	97.25
Melting point, °C	60.5	46.0	53.5	49.8
Refractive index at 55°C	1.4324	1.4377	1.4463	1.4420
Acid value	201.7	153	203.2	173.8
Saponification value	270.0	178	218	207.4
Volatile acids, *per cent*	None.	None.	0.58	
Hydroxy stearic acids, *per cent*	15.80	7.2	25.4	15.21
Neutral fats, *per cent*	1.37	6.5	25.8	15.3
Iodine value	6.04	7.4	18.2	13.44
Acetyl "	34.75			22.71

THE DETERMINATION OF AMMONIA IN THE BLOOD WITH THE AID OF PERMUTIT.

By S. MORGULIS and M. JAHR.

TITRATION OF THE BICARBONATE IN BLOOD PLASMA.

By DONALD D. VAN SLYKE and EDGAR STILLMAN.

DEHYDRATED VEGETABLES FOR ARMY USE.

By S. C. PRESCOTT.

THE ACTION OF ANTAGONISTIC ELECTROLYTES ON THE CONDUCTIVITY OF EMULSIONS AS COMPARED WITH PROTOPLASM.

By G. H. A. CLOWES and F. WEST.

APPLICATION OF THE PRINCIPLES OF NUTRITION IN AN ARMY CAMP.

By R. J. ANDERSON.

THE ARMY RATION IN FRANCE.

By PHILIP A. SHAFFER.

MILITARY HOSPITAL DIETARIES.

By R. G. HOSKINS.

THE ACID-BASE BALANCE OF FOOD CONSUMED IN ARMY CAMPS.

By N. R. BLATHERWICK.

A PHYSICOCHEMICAL METHOD OF CHARACTERIZING PROTEINS.

By L. J. HENDERSON and E. J. COHN.

ON THE CHEMICAL CONSTITUTION OF YEAST NUCLEIC ACID.

By WALTER JONES.

ARGININE AND ARGINASE IN THEIR RELATION TO THE PRODUCTION OF CREATINURIA.

By H. STEENBOCK, E. D. GROSS, and A. KOEHLER.

THE DETERMINATION OF NITROGEN IN DROP QUANTITIES OF BLOOD BY DIRECT NESSLERIZATION.

By AMOS W. PETERS.

THE FUNCTIONAL CAPACITY OF THE KIDNEYS AND THE BLOOD FINDINGS IN LATE CASES OF "TRENCH NEPHRITIS."

By A. P. LOTHROP and W. T. CONNELL.

PROCEEDINGS OF THE AMERICAN SOCIETY OF BIOLOGICAL CHEMISTS.

FOURTEENTH ANNUAL MEETING.

Cincinnati, Ohio, December 29–31, 1919.

THE ETIOLOGY OF RICKETS.

By E. V. McCOLLUM, NINA SIMMONDS, and HELEN T. PARSONS.

(*From the Laboratory of Chemical Hygiene, School of Hygiene and Public Health, the Johns Hopkins University, Baltimore.*)

We have conducted an extensive series of experiments with rats restricted to diets derived from cereals and legume seeds; cereals, legume seeds, and muscle meats; and with similar diets in which degerminated products of cereal grains replaced whole seeds; and have supplemented these mixtures with purified food substances to determine the nature and extent of their dietary shortcomings.

In these experiments we have observed the gross picture of rickets in many of the animals restricted to faulty diets, and have demonstrated that this condition develops on diets in which the faults lie in several different factors.

A low content of fat-soluble A, low calcium content, poor quality of protein, and unsatisfactory salt combinations, acting in combinations, may all contribute to the etiology of the disease. We have not yet completed our observations on diets in which but a single factor is at fault. It is certain that specific fasting for fat-soluble A cannot be regarded as the sole and only possible cause of rickets.

Since the same gross picture can be induced in several different ways, we are led to suggest the possible occurrence of more than one kind of rickets. Histological studies of tissues of animals suffering from what appears to be rickets, but from different causes, are still in progress. No decision can yet be reached as to whether in all cases the histological picture is the same in animals exhibiting beaded ribs, enlargements of the costochondral junctions, deformity of the thorax, and general deformity of the body, irrespective of the dietary factor or factors which brought about the condition.

THE RÔLE OF FAT-SOLUBLE VITAMINE IN HUMAN NUTRITION AND ITS SUGGESTED RELATION TO RICKETS.

By ALFRED F. HESS.

(From the Bureau of Laboratories, Department of Health, New York.)

It has been shown that the fat-soluble vitamine is an essential constituent of the dietary of rats. There have also been clinical reports attributing marked malnutrition in infants and children to a lack of this dietary factor (Japan, Denmark). As a result of these experiences it has been accepted that this vitamine is highly important for man, and that the lack of it leads to nutritional disorder in children. This has been emphasized all the more as this vitamine is not nearly so widely distributed in nature as is the water-soluble vitamine. In order to study this question five infants, varying in age from 5 to 12 months, were given a diet which was complete except for a very small amount of fat-soluble vitamine. It consisted of 180 gm. daily of highly skimmed milk ("Krystalak" 0.2 per cent fat), 30 gm. of cane sugar, 15 to 30 gm. of autolyzed yeast (to supply water-soluble vitamine), 15 cc. of orange juice, 30 gm. of cottonseed oil, and cereal for the older infants.

On this diet the children have done well for a period of 8 to 9 months. They have shown no anemia, no eye trouble, no bone changes, as seen by the x-ray, nor has their growth in length or in weight suffered. We believe, therefore, that either a very small amount of this vitamine suffices to supply the needs of human nutrition, or that this deficiency has to be maintained for a period of years in order to bring about any harmful result. Danger from a lack of this dietary factor need not be apprehended if the diet is otherwise complete.

The development of rickets has been attributed by Mellanby, as a result of experiments on dogs, to a lack of fat-soluble vitamine, and Hopkins and Chick have termed this vitamine the "anti-rachitic factor." It was found, however, that infants fed on this "fat-soluble vitamine minimal diet" did not develop the well established signs of rickets—beading of the ribs, enlargement of the epiphyses, weakness of the muscles, etc. We cannot believe, therefore, that rickets is brought about merely by a deficiency of this principle; all the more so, as this disorder de-

veloped in infants receiving large quantities of milk containing ample fat-soluble vitamine. It may be added that neither cream nor the leafy vegetables, both of which are rich in this principle, are comparable to cod liver oil as growth stimulants.

PRELIMINARY OBSERVATIONS ON THE RELATION OF BACTERIA TO EXPERIMENTAL SCURVY IN GUINEA PIGS.

By MAURICE H. GIVENS and GEORGE L. HOFFMANN.

(From the Research Laboratories, Western Pennsylvania Hospital, Pittsburgh.)

Whether or not bacteria play any rôle in the development of scurvy in guinea pigs has not been settled by direct evidence. Jackson and Moore[1] found coccus-like bodies in microscopic sections of lesions in scorbutic guinea pigs. Jackson and Moody[2] isolated from the diseased joints, muscles, and lymph glands of these animals Gram-positive and Gram-negative organisms. Pure strains of these bacteria introduced into guinea pigs gave rise in most instances to hemorrhagic and other lesions in the bones, joints, muscles, lymph glands, and organs. Torrey and Hess[3] concluded that scurvy, both of guinea pigs and of infants, was not associated with an overgrowth of putrefactive bacteria in the intestinal tract.

We have attempted to throw further light upon the question by bacteriological examinations of the blood, joints, and feces of guinea pigs made scorbutic on different diets and then treated with different antiscorbutic foods. Blood from scorbutic animals anesthetized and from those dying of the disease regardless of the diet producing the same has been found to be sterile. The enlarged front joints of guinea pigs developing scurvy on oats alone were sterile; this was likewise true in the majority of cases of guinea pigs developing scurvy on the soy cake food of Givens and Cohen.[4] However, in two or three instances a staphylococcus and diplococcus were isolated. Pure strains of these organisms

[1] Jackson, L., and Moore, J. J., *J. Infect. Dis.*, 1916, xix, 510.

[2] Jackson, L., and Moody, A. M., *J. Infect. Dis.*, 1916, xix, 511.

[3] Torrey, J. C., and Hess, A. F., *Proc. Soc. Exp. Biol. and Med.*, 1917–18, xv, 74.

[4] Givens, M. H., and Cohen, B., *J. Biol. Chem.*, 1918, xxxvi, 127.

injected intracardially, intraperitoneally, and into the joints of healthy guinea pigs on a mixed diet produced no signs of scurvy. Smears and cultures were made of material from different parts of the intestinal tract of guinea pigs on oats alone, on oats plus lemon juice, 3 cc. daily, after scurvy developed, on the soy cake diet, and on the same plus cabbage after the appearance of scurvy. No marked difference was found in the intestinal flora under any of these conditions.

FURTHER STUDIES ON THE USE OF WATER-SOLUBLE B IN THE TREATMENT OF INFANT MALNUTRITION.

By WALTER H. EDDY.

(From the Society of the New York Hospital, New York.)

Results of experiments were reported confirming previous work[5] of the author in stimulating growth by the addition of B vitamine extract to the diet of infants suffering from malnutrition of the marasmus type. A new feature used in the study was the application of the Bachmann test[6] to measurement of dosage.

In experiments with vitamine prepared from the navy bean by the McCollum method[7] the test detected relatively small amounts of vitamine and, while in need of further standardization, offered a valuable aid in measurement of the vitamine B present in the substances used. Tables were shown giving the result of the test on various amounts of the dextrin-vitamine mixture and on other substances such as milk, both cow and human milk.

The first case, showing stimulation with the B vitamine, gained an average of 0.84 ounces per day in a 32 day period as against a gain of 0.47 ounces per day during a 17 day period preceding the use of the vitamine through the calorie intake and the food given remained constant through both periods. The second case showed a similar stimulation though not so well controlled as the first. The interesting feature of the use of the Bachmann test as applied to the first case was the result of the tests as applied to the child's diet and to the extract. The diet

[5] Eddy, W. H., and Roper, J. C., *Am. J. Dis. Child.*, 1917, xiv, 189.
[6] Bachmann, F. M., *J. Biol. Chem.*, 1919, xxxix, 235.
[7] McCollum, E. V., and Simmonds, N., *J. Biol. Chem.*, 1918, xxxiii, 55.

was found to contain 2,120 units of vitamine and the stimulating mixture only 70 units.

In other words an increase of only 3 per cent in actual vitamine intake produced the marked stimulation. The author suggested that this result may be due to the fact that the child could utilize the extracted vitamine when it could not utilize the vitamine in the diet and that the way the vitamine is held in a diet may be an important factor. In all the baby cases treated the extract feeding is followed by an increased growth which continued to a point where removal of the extract is possible without marked reduction in the growth rate and the child then goes on to recovery. These cases represent the fifteenth and sixteenth showing stimulation under this treatment.

THE EXOGENOUS AND ENDOGENOUS NATURE OF THE CREATINURIA IN THE GROWING DOG.

By VICTOR JOHN HARDING and ELRID G. YOUNG.

(*From the Biochemical Laboratory, McGill University, Montreal.*)

In investigating the creatinuria of pups, and studying the effect of high and low protein diets upon that condition, the authors find in most of the animals that the creatine excretion is dependent upon the level of protein intake. Only in one animal, however, did they find that the creatinuria could be totally abolished by a low protein diet, and even in this case creatine again made its appearance in the urine in spite of the low protein diet. In most of the animals investigated there always remained a residuum of creatine in the urine, which invariably increased on a continuation of the low protein diet. This increase in the creatine excretion, following its lowering with a low protein diet, is ascribed to the production of that substance from some store of intermediary metabolites. The authors are thus of the opinion that creatine can be of both exogenous and endogenous origin and that both can exist in the same animal. The endogenous creatine is not a constant quantity, and its amount is determined by factors as yet unknown. In two animals, however, the authors were unable to affect the excretion of creatine by an alteration in the protein of the diet. In these animals apparently the endogenous fraction is so large as to obscure the exogenous portion.

CYSTINE AS THE PRECURSOR OF CREATINE IN THE GROWING DOG.

By VICTOR JOHN HARDING and ELRID G. YOUNG.

(*From the Biochemical Laboratory, McGill University, Montreal.*)

The authors have investigated the effects upon the exogenous creatine excretion in pups of variations in the amino-acid content of the protein.

Arginine is without any effect. A variation in the content of the cystine in the protein or the addition and withdrawal of that amino-acid as such is followed by a variation in the creatine excretion. It is found, however, that as well as affecting the exogenous function of the creatine the endogenous portion is also disturbed.

The hypothesis has been formulated that cystine forms creatine through the intermediate stages of taurine and amino ethyl alcohol, followed by methylation, combination with urea, and oxidation.

THE ORIGIN OF CREATINE.

By R. B. GIBSON and FRANCES T. MARTIN.

(*From the Chemical Research Laboratory, Department of Theory and Practice of Medicine, the State University of Iowa, Iowa City.*)

Ingested creatine is promptly eliminated chiefly as creatine, in part as creatinine, in cases of advanced progressive pseudohypertrophic muscular dystrophy. The creatine and to a lesser extent the creatinine are increased with a high protein intake inasmuch as in the case reported half again as much creatinine and twice as much creatine may be excreted as on a low nitrogen plane. This increase is obtained from the catabolized protein (including gelatin) and not from that retained for growth purposes. The substitution of the arginine-rich protein edestin for the protein of the diet does not affect the creatine output. Ingested asparagine and sarcosine do not lead to increased creatine formation; glycocyamine is converted in part (at least 36 per cent) in a controlled experiment, thus confirming earlier work. It is probably not a stage in the ordinary creatine formation.

CORRELATION OF CERTAIN PHYSICAL AND CHEMICAL FACTORS WITH TOXICITY TO MARINE ORGANISMS.

By G. H. A. CLOWES and L. G. KEITH.

(From the Biochemical Research Laboratory, Eli Lilly and Company, Indianapolis.)

The absolute and relative toxicities of the symmetrical and asymmetrical dichloroacetones to developing sea urchin eggs correspond with their toxicities to mice, the symmetrical compound being sixty times as toxic as the asymmetrical.

Experiments with mustard gas and other war gases have indicated that their relative toxicities depend upon rate of hydrolysis, lipoid water distribution coefficient, vapor tension, etc. The dichloroacetone experiments support this view. The symmetrical compound diffuses more rapidly from a non-aqueous to an aqueous phase, and hydrolyzes more rapidly in a feebly alkaline aqueous phase than does the asymmetrical.

With both substances a latent period was observed, during which the gases could be removed by shaking with additional eggs or charcoal, the protective effect depending upon temperature, time of application, and amount of protective substance employed.

An analysis of the data obtained by exposing varying numbers of eggs to varying concentrations for varying times at varying temperatures, and subjecting them to varying protective procedures, supports the view previously advanced that the war gases are first adsorbed on the surface of the protoplasmic structure, then penetrate through protoplasm by diffusion, and finally after a latent period undergo hydrolysis causing the death of the cell.

METABOLISM OF O-NITROBENZALDEHYDE, M-NITROBENZALDEHYDE, AND P-NITROPHENYLACETALDEHYDE.

By CARL P. SHERWIN, JOHN A. DALY, and WALTER A. HYNES.

(From the Research Laboratory, Fordham University, New York.)

According to the investigations of Cohn, *o*-nitrobenzaldehyde, when fed to the rabbit, is mostly destroyed, and only about 10 per cent is excreted in the urine as *o*-nitrobenzoic acid. *m*-Ni-

trobenzaldehyde is excreted by the rabbit as *m*-acetylaminobenzoic acid, and by the dog as *m*-nitrohippuric acid urea. *p*-Nitrobenzaldehyde is excreted by the rabbit as a combination of *p*-nitrobenzoic acid and *p*-acetylaminobenzoic acid, and by the dog *p*-nitrobenzaldehyde is excreted in the urine as *p*-nitrohippuric acid urea.

We fed 2 gm. of *o*-nitrobenzaldehyde to a man and recovered 65 to 70 per cent of *o*-nitrobenzoic acid from the urine. No reduction of the nitro group was apparent in this case, nor was there any combination with glycocoll by the *o*-nitrobenzoic acid thus produced.

m-Nitrobenzaldehyde was fed to a man in 5 and 6 gm. doses. From the urine we recovered about 75 per cent of the substance in the form of *m*-nitrobenzoic acid, and after the 6 gm. dose a fraction of a gm., about 5 per cent, was found as *m*-nitrohippuric acid.

p-Nitrophenylacetaldehyde was fed to a rabbit in 1 gm. doses, and 75 to 80 per cent was excreted as *p*-nitrophenylacetic acid, while *p*-nitrophenylacetic acid, when fed, resulted in rapid elimination of the same substance in the urine. 5 gm. of *p*-nitrophenylacetaldehyde were also fed to a dog and excreted in the urine as *p*-nitrophenylacetic acid. *p*-Nitrophenylacetic acid, as previously shown by one of us, is excreted mostly as the same substance in the urine of the dog, but a small fraction was also excreted in combination with glycocoll as *p*-nitrophenaceturic acid. 5 gm. of *p*-nitrophenylacetaldehyde were ingested by a man and 70 per cent of this was recovered from the urine as *p*-nitrophenylacetic acid. *p*-Nitrophenylacetic acid, as we have previously shown, is excreted by the human uncombined.

In these cases we could find no reduction of the nitro group in any of the aldehydes, as found by Cohn in his work on rabbits, so we fed 5 gm. of *p*-aminophenylacetic acid to a human, and again to a dog. In both cases there appeared in the urine a dark red substance, easily soluble in all organic solvents and insoluble in water. This substance has not as yet been crystallized or identified.

RELATION BETWEEN AMMONIA EXCRETION AND THE HYDROGEN ION CONCENTRATION OF URINE.

By CYRUS H. FISKE.

(From the Biochemical Laboratory, Harvard Medical School, Boston.)

Hasselbach's supposed constant relation between the ammonia coefficient and the C_H of urine is subject to deviations of sufficient frequency to suggest that other controlling factors are concerned. An examination has consequently been made, on hourly samples of human urine, of (1) the C_H, and (2) a ratio, that is more likely than the ammonia coefficient to have a real significance in connection with the regulation of reaction; *viz.*, the ratio of ammonia to sulfuric acid. Other things being equal, this ratio rises with the C_H, but the relation is by no means constant under all conditions. At the same C_H, the ratio is lower the higher the sulfate content, and also the higher the phosphate content. The dependence on the sulfate excretion may be explained by the fact that acids, other than sulfuric, that call for the formation of ammonia, are relatively less in amount the greater the rate of production of sulfuric acid, while the dependence on the phosphate output is perhaps to be accounted for by the supposition that the demand for ammonia is less the greater the amount of phosphate available for excretion, since the conversion of secondary to primary phosphate liberates fixed alkali.

THE AMINO-ACID CONTENT OF BLOOD FROM THE JUGULAR AND MAMMARY VEINS OF MILKING COWS.

By C. A. CARY.

(From the Research Laboratories of the Dairy Division, Bureau of Animal Industry, United States Department of Agriculture.)

An attempt was made to determine the change in the amino-acid content of blood and of blood plasma as it passes through the mammary gland of milking cows by comparing the amino-acid N in samples obtained as nearly simultaneously and with as little disturbance as practical from the jugular and mammary (abdominal subcutaneous) veins.

The proteins were removed by the coagulation-trichloroacetic acid-kaolin method described by Bock,[8] the amino N being determined by the HNO_2 method, using the smaller apparatus of Van Slyke. The urea, etc. were corrected for by making duplicate runs allowing a longer time for deamination.[9]

The averages of duplicate analyses of plasma are given in Table I.

TABLE I.

Vein.	Amino-acid content of 100 cc. of blood plasma.					
	I	II	III	IV	V	VI
	mg.	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*
Jugular	2.68	3.31	2.41	2.49	2.38	2.47
Mammary	1.78	2.34	2.45	1.93	2.00	1.87
Difference	0.90	0.97	0.04	0.56	0.38	0.60

The cows gave about 10 kilos of milk daily. The plasma constituted about 67 per cent by volume of the whole blood. Using a very rough approximation[10] of the flow of blood through the mammary gland, assuming that the difference in amino-acid content of the mixed arterial and jugular bloods is relatively negligible, and that the changes in the plasma thus indicated are effected continuously throughout the 24 hours, our largest differences indicate that about 35 gm. of amino-acid N are abstracted daily from the plasma passing through the gland. This estimate is rough and it is very doubtful whether we get samples of blood indicating the maximum changes occurring in the gland. It is, however, suggestive relative to the formation of milk proteins.

With the whole blood the differences are in general in the same direction as those found in the corresponding plasma, but more work must be done to determine whether there is any change or not in the amino-acid N of the corpuscles of blood perfusing the gland. The work is still in progress.

[8] Bock, J. C., *J. Biol. Chem.*, 1916–17, xxviii, 357.

[9] Van Slyke, D. D., and Meyer, G. M., *J. Biol. Chem.*, 1912, xii, 399.

[10] Meigs, E. B., Blatherwick, N. R., and Cary, C. A., *J. Biol. Chem.*, 1919, xxxvii, 21.

NEUTRALITY REGULATION IN CATTLE.

By N. R. BLATHERWICK.

(From the Research Laboratories of the Dairy Division, Bureau of Animal Industry, United States Department of Agriculture.)

The alkaline reserve of the plasma of cows, as measured by the CO_2 capacity, is remarkably constant. The average normal value, 60.9 cc. of CO_2 per 100 cc. of plasma, is somewhat lower than that of normal human plasma. Maximum and minimum values were 65.5 and 55.1 cc., respectively. The CO_2 capacity of the plasma of three pregnant cows showed no evidence of the acidosis of pregnancy observed by others in women. By feeding a cow on rations restricted to one food, namely hay, grain, or silage, changes in plasma CO_2 capacity and in the ammonia and CO_2 of the urine were produced. A cow fasted for 7 days failed to show a decrease in the CO_2 capacity of the plasma, but rather a slight increase apparent at 24 hours, which was maintained throughout the fast. Coincident with this was an increase of 11 per cent in the inorganic P of the plasma. Larger increases in the inorganic P of the plasma, as the result of fasting, have been found in this laboratory. This change indicates a mobilization of bone substance to assist in the maintenance of neutrality. Another important factor in maintaining the correct reaction of the cow's body is the excretion of CO_2 in the urine. This has been observed to vary from 6 cc. per 100 cc. in the urine of a young calf living on a milk diet to 394 cc. in that of a cow eating silage, grain, and hay.

EFFECT OF ACIDS, ALKALIES, AND SALTS UPON CATALASE PRODUCTION.

By W. E. BURGE.

(From the Department of Physiology, University of Illinois, Urbana.)

100 cc. of 0.15 per cent hydrochloric acid decrease the catalase of the blood about 30 per cent in 2 hours, while a similar quantity of acetic acid increases it about 14 per cent. The decrease produced by the hydrochloric acid is due in part to the destruction of the enzyme and in part to the inhibiting action of the acid; the increase produced by the acetic acid is due to the stimulation

of the alimentary glands, particularly the liver, to an increased output of the enzyme. Carbonic acid was found to have no effect on the catalase of the blood. 10 gm. per kilo of sodium carbonate as well as sodium acetate dissolved in 100 cc. of water increase the catalase of the blood. The animals used in these experiments were rabbits and the substances were introduced by means of a stomach tube.

A STUDY OF THE DISTRIBUTION OF CATALASE IN THE KIDNEY.

By SERGIUS MORGULIS and VICTOR E. LEVINE.

(*From the Department of Biochemistry and Physiology, College of Medicine, Creighton University, Omaha.*)

Experiments with the kidney of rabbit, sheep, pig, and beef, perfused with saline and unperfused, indicate a catalase content (quantity of hydrogen peroxide decomposed) and a catalytic activity (rate of decomposition) which are much greater in the cortex than in the medulla. The upper portion of the medulla yields greater values than the papillary portion. Part of the catalase is derived from the blood and can be removed by perfusion; part remains insoluble even after prolonged perfusion. Results with the right and the left kidney from the same animal point to the fact that the two organs differ in catalase content and catalytic activity. The kidney tissue contains no direct oxidizing enzyme. Quantitative experiments on peroxidase show that the peroxidase relations are the opposite of those of catalase. The greatest peroxidase content and peroxidase activity (rate of reaction) are found in the medulla; the cortex values are comparatively small. Perfusing the kidney results in the complete removal of peroxidase, although catalase is removed only in part by this procedure.

THE SIGNIFICANCE OF CONCENTRATION WITH REFERENCE TO SUBSTANCES IN THE BLOOD PLASMA.

By R. T. WOODYATT.

(From the Laboratory of Clinical Research, Rush Medical College, the Otho S. A. Sprague Memorial Institute, Chicago.)

A dog weighing 10 kilos received glucose by vein constantly for 8 hours at the uniform rate of 10 gm. per hour. In the first 4 hours the glucose was given in dilute solution; in the second 4 hours in concentrated solution; *i.e.*, first in about 3 and later in about 36 per cent form. The dog passed in the urine 0.1 to 0.2 gm. of sugar per hour throughout (except in the first hour). In the 2nd, 3rd, 4th, and 5th hours the blood sugar percentage was 0.13 to 0.14 per cent. In the 6th, 7th, and 8th hours it was 0.21 to 0.23 per cent. This change followed the change in the volume of water injected and occurred while the rates of glucose injection, glucose elimination, and hence glucose utilization all remained constant. If anything, the glucose excretion was a trifle higher during the period of low blood sugar percentages. The experiment is one of a group and depicts a type.

The phenomenon was discussed in relation to the observations of Magnus on salts; and Epstein on the blood sugar and rate of glycosuria in diabetes. It was pointed out that if the surface of contact between the blood and the cells should vary in direct proportion to the blood volume, the results would be explained. Anatomical details of capillary systems were discussed to show how an increasing volume of capillary blood—especially by opening up erstwhile empty capillary collaterals—may develop surface approximately in proportion to the volume of extra blood.

In interpreting the physiological effects of a substance in the blood plasma, the factor of concentration alone is not sufficient. Surface is also important. Possibly during normal variations of the blood volume, surface and volume vary in direct proportion.

ALKALOID DIFFUSION IN PHYSICAL AND BIOLOGICAL SYSTEMS.

By G. H. A. CLOWES and A. L. WALTERS.

(*From the Biochemical Research Laboratory, Eli Lilly and Company, Indianapolis.*)

Alkaloids adsorbed by fullers' earth cannot be released by extraction with water or an aqueous solution of sodium bicarbonate having the alkalinity of intestinal contents. Nevertheless when the alkaloid adsorption compound is administered by mouth, the alkaloid is adsorbed by the intestinal mucosa. It was found, for example, that adsorbed atropine administered to cats by mouth produced a prolonged dilatation of the pupil of the eye. Adsorbed strychnine caused convulsions and death in rabbits and rats, and adsorbed quinine and emetine were recoverable in the urine. Adsorbed strychnine introduced into the peritoneal cavity caused convulsions and death, but when the adsorbed strychnine was first introduced into a collodion sac which was freely permeable for strychnine but would not permit of the passage of cells, and the sac introduced into the peritoneal cavity, there was no effect.

These experiments show that the alkaloid may be extracted by protoplasm in a neutral medium and suggest the probability that the alkaloid is released by direct contact between the protoplasmic material of the cell and the adsorption compound.

In the above experiments from two to ten times the amount of adsorbed alkaloid was required to produce a result equivalent to that obtained in control experiments with free alkaloid. Alkaloids were extracted from their adsorption compounds to a slight extent by intestinal contents, somewhat better by means of intestinal mucosa, egg yolk, etc., but with considerable facility by so called neutral soaps and soaps to which either fatty acid or alkali had been added. The extracts were carried out either in aqueous or mixed aqueous organic media or in emulsions using soaps of sodium, potassium, calcium, and magnesium.

These results support the view that soaps and the fatty acid group play an important rôle in protoplasmic assimilation, and explain the therapeutic effects obtained by administering alkaloids in the adsorbed form.

THE NATURE OF THE LIGHT-PRODUCING REACTION OF LUMINOUS ANIMALS.

By E. NEWTON HARVEY.

(From the Physiological Laboratory, Princeton University, Princeton.)

Animal luminescence results from the oxidation of a substance, luciferin (LH_2), in presence of a catalyst, luciferase, water, and oxygen. When solutions of luciferin and luciferase are mixed, not enough CO_2 is produced to saturate the buffers in these solutions and probably no CO_2 at all is produced; not enough heat is produced to raise the temperature of the mixture 0.001°C., and from this figure the heat of oxidation of luciferin is calculated to be less than 0.1 calorie per gm. The oxidation product of luciferin, oxyluciferin (L), is very similar to luciferin and may be reduced to luciferin again by various perhydridases and by nascent hydrogen or H_2S. The reduction is accelerated by acid and in the presence of light. Thus the reaction is photogenic in one direction, photochemical in the opposite direction, and may be represented as follows:

$$\text{Luciferin } (LH_2) + O \underset{\text{Darkness. Alkali. Luciferase.}}{\overset{\text{Light. Acid. Perhydridase or nascent H.}}{\rightleftarrows}} \text{oxyluciferin } (L) + H_2O$$

THE INFLUENCE OF OXYGEN DEFICIENCY AND RELATED CONDITIONS UPON THE HEMATO-RESPIRATORY FUNCTIONS.

By YANDELL HENDERSON and HOWARD W. HAGGARD.

(From the Physiological Laboratory, School of Medicine, Yale University, New Haven.)

It is generally supposed that under oxygen deficiency acids are produced in the tissues and are retained in the blood, and that the blood alkali is thus neutralized and eliminated through the urine. This may be termed the acidotic process.

Experiments on dogs subjected to progressively decreasing oxygen show, however, that the process actually involved is in many respects exactly the opposite of the usual supposition. They demonstrate that before any considerable amount of alkali is lost an abnormally large amount of CO_2 is eliminated by the excessive breathing induced by a lowered oxygen pressure in the air breathed. Then alkali passes out of the blood to compensate this alkalosis. This we term the acapnial process.

From these facts and related observations on men it appears that in normal persons the blood alkali is controlled by the dissolved CO_2—more or less alkali being called into use in the blood to satisfy the normal $H_2CO_3 : NaHCO_3$ relation, and thus to keep the C_H of the blood nearly constant. The amount of dissolved CO_2 in the blood is controlled by the pulmonary ventilation, and fundamentally in normal persons the ventilation is adjusted to the oxygen partial pressure of the altitude at which the person lives.

When, however, overbreathing is induced and the H_2CO_3 of the blood is reduced, the $NaHCO_3$ follows downward. This, we find, and not acidosis, is what occurs also in carbon monoxide asphyxia.

A simple test for differentiating between low blood alkali of acidotic and that of acapnial origin is the administration of air to which 8 to 10 per cent of CO_2 has been added. This quickly causes death in acidotic subjects; but it induces a rapid recall of alkali to the blood, and restoration of the subject to a virtually normal condition of health if the condition is of acapnial origin.

We have now (in collaboration with Dr. R. C. Coburn) applied this treatment to patients after surgical operation and anesthesia. The result has been that alkali was recalled to the blood, that arterial pressure and other functions were restored to normal, and that the anesthetic was rapidly eliminated by the full breathing during the CO_2 inhalation with a consequent great reduction of nausea.

In dogs asphyxiated with carbon monoxide (in investigations for the United States Bureau of Mines) strikingly beneficial results have been obtained by means of oxygen reenforced with 10 per cent CO_2.

RELATION OF ANESTHESIA TO RESPIRATION.

By SHIRO TASHIRO.

(*From the Biochemical Laboratory, College of Medicine, University of Cincinnati, Cincinnati.*)

The rate of the tissue respiration of the claw nerves of various crabs is highest in lobster, then spider crab, and least in *Limulus*. That of the sciatic nerve of frog comes between the last two. The time required for complete anesthesia with the same narcotics is shortest for lobster, then spider crab, frog, and *Limulus* in order. Susceptibility of the nerve to anesthetics runs parallel to the rate of its respiration, provided that other factors are fairly constant. A 0.4 per cent solution of chloral hydrate does not alter the original gradient of respiration of the spider crab's nerve, but 2 per cent, which produces reversible loss of irritability, reverses it. These results can be best explained if we accept the idea that susceptibility of the same nerve toward anesthetics is quite different along the fiber, depending mainly upon the different respiratory activities of the different parts of the fiber. The advantage of using tissue with as few functions as possible is obvious, for we must know exact physiological conditions of all parts of the tissue when we measure the respiration. Abolition of one function in a tissue may or may not mean complete anesthesia of the tissue as a whole. It is suggested that primary stimulation by weak concentrations of narcotics is probably caused by exaggerating the original metabolic gradient; and final anesthesia, by further oxidative interference which primarily changes the relation between tissue respirations of the different parts of the tissue.

DETERMINATION OF METHEMOGLOBIN IN BLOOD.

By W. S. McELLROY.

(*From the Laboratory of Physiological Chemistry, School of Medicine, University of Pittsburgh, Pittsburgh.*)

When methemoglobin is present the colorimetric determination of hemoglobin cannot be used as an index of the oxygen-carrying capacity of the blood. Under these conditions the oxygen capacity must be determined directly.

Methemoglobin interferes with the colorimetric determination of hemoglobin as carbon monoxide hemoglobin and acid hematin.

The total hemoglobin in the presence of methemoglobin can be determined colorimetically by converting the oxyhemoglobin into methemoglobin by means of potassium ferricyanide or other suitable reagent and determining the total as methemoglobin.

The oxygen capacity gives the amount of oxyhemoglobin.

The difference between the total hemoglobin determined as methemoglobin and the oxyhemoglobin estimated from the oxygen capacity gives the amount of methemoglobin.

BLOOD PHOSPHATES IN THE LIPEMIA PRODUCED BY SEVERE HEMORRHAGE.

By W. R. BLOOR and E. D. FARRINGTON.

(*From the Department of Biochemistry and Pharmacology, University of California, Berkeley.*)

Changes directly connected with the lipemia were confined to increases in lipoid phosphorus in plasma (up to five times the normal value) and in corpuscles (double the normal value).

Changes attributable to the bleeding and regeneration were mainly increases in the inorganic phosphates in the corpuscles and, to a less extent, changes in the quantities of the other phosphoric acid combinations.

FAT CONTENT OF EMBRYONIC LIVERS.

By C. G. IMRIE and S. G. GRAHAM.

(*From the Laboratory of Pathological Chemistry, University of Toronto, Toronto.*)

Observations were made upon the fat content of the livers of embryonic guinea pigs throughout the period of gestation. Such changes, if any, had first to be established before the relation of the embryonic liver to fat mobilized from the storehouses in the pregnant animal could be interpreted. The results of this preliminary investigation were reported. The higher fatty acid content was determined by saponification and extraction, as described by Leathes, and the iodine value by the method of Wij. The weight of the embryo was employed as an indication

of its age. Until the embryo reaches a stage in its development corresponding to a weight of 35 to 40 gm., the fat content of the liver more or less closely approximates that of the maternal liver, which is from 2 to 3 per cent. After this period, however, there is a progressive accumulation of fat in the embryonic liver, so that at birth the animal has from 16 to 18 per cent of higher fatty acids reckoned upon the moist tissue. The iodine value of this fat was comparatively high, though lower than that in the maternal liver.

A study of the fate of this fat showed that it is rapidly utilized by the animal during the first 48 to 72 hours of its life. As the fat content is lowered during this period, the iodine value rises.

Figures and charts representing these changes were presented.

QUANTITATIVE COLORIMETRIC DETERMINATION OF TYROSINE AND TYRAMINE (P-HYDROXYPHENYLETHYLAMINE) AND OTHER PHENOLS.

By MILTON T. HANKE and KARL K. KOESSLER.

(From the Otho S. A. Sprague Memorial Institute and the Departments of Pathology and Physiological Chemistry, University of Chicago, Chicago.)

A method has been developed for estimating tyrosine, tyramine *p*-cresol, oxyphenylacetic acid, and phenol by means of which quantities as small as 0.000005 gm. of these substances can be determined. The method is based upon the interaction, in alkaline solution, of the phenols with *p*-phenyldiazonium sulfonate. The color so obtained is intensified and stabilized by the addition of NaOH and hydroxylamine hydrochloride. The theory of the reaction involving the stabilization of the quinone ring by the hydroxylamine was discussed in detail.

QUANTITATIVE COLORIMETRIC DETERMINATION OF HISTIDINE AND HISTAMINE IN PROTEINS AND PROTEIN-CONTAINING MATTER.

By KARL K. KOESSLER and MILTON T. HANKE.

(From the Otho S. A. Sprague Memorial Institute and the Departments of Pathology and Physiological Chemistry, University of Chicago, Chicago.)

A method has been developed for estimating histidine and histamine in proteins and protein-containing matter that is based upon the method developed for pure imidazoles and previously described by us,[11] and upon the method developed by Van Slyke for the estimation of the hexone bases.[12]

DIGESTIBILITY OF RAW CORN-STARCH.

By C. F. LANGWORTHY and HARRY J. DEUEL, Jr.

(From the Office of Home Economics, States Relation Service, United States Department of Agriculture.)

Raw starches are generally considered to be digested only slightly, if at all, by the human body. In the experiments here reported the digestibility of raw corn-starch when eaten in quantity by normal men was determined. There were some variations, but on an average the three subjects ate 250 gm. of raw corn-starch a day during the 3 day experimental period, in the form of a frozen custard, in which the starch was combined with milk, sugar, and a little salt, and flavored with lemon extract to mask the "starchy" taste. The methods were those usually followed in experiments with cereals carried on in this laboratory. The total amount of feces pertaining to the experimental period was very small and on examination did not show any starch.

The digestibility of the raw corn-starch was found to be 100 per cent. So far as could be seen, its ingestion had no abnormal physiological effects and the subjects remained in their accustomed good health.

The experiments here reported are the first of a series dealing with the digestibility of raw starches, in which it is planned to include potato, rice, wheat, arrowroot, and possibly other starches.

[11] Koessler, K. K., and Hanke, M. T., *J. Biol. Chem.*, 1919, xxxix, 521.
[12] Van Slyke, D. D., *J. Biol. Chem.*, 1911–12, x, 15.

SPERM AS AN INHIBITANT OF MATURATION AND FERTILIZATION.

By G. H. A. CLOWES and ESTHER GREISHEIMER.

(From the Biochemical Research Laboratory, Eli Lilly and Company, Indianapolis.)

Immature starfish eggs are prevented from maturating by treatment with butyric acid, exposure to heat, etc., the identical procedures which, after maturation, induce artificial parthenogenesis.

Similarly exposure of the immature eggs to sperm, even in small amounts, inhibits or retards maturation rendering the eggs unfertilizable or difficultly fertilizable by sperm, at a subsequent stage at which control eggs not previously treated with sperm are readily fertilized.

The best contrasts may be obtained by giving the immature eggs a preliminary exposure to a weak butyric acid solution which retards but does not prevent maturation. This treatment followed immediately by exposure to sperm causes a marked interference with maturation and subsequent fertilization as compared with eggs which receive the butyric treatment only. Heat followed by sperm gives similar results.

Inhibiting effects of sperm on immature eggs may be counteracted by a brief treatment with 0.001 N NaOH, but not ammonia. The similarity of inhibiting effect obtained by butyric acid and sperm separately or in combination, and the reversal of these effects by NaOH, suggest that inhibiting effect of sperm is attributable to production of acid on or adjacent to egg surface.

COLLOID CHEMISTRY OF SOAPS AND PROTEINS.

By MARTIN H. FISCHER.

(From the Physiological Laboratory, University of Cincinnati, Cincinnati.)

On the assumption that the polymerized amino-acids which we call proteins react with bases of various sorts to form salts in the same fashion in which fatty acids unite with bases of various sorts to form soaps, the colloid chemistry of the latter was studied to obtain light regarding the colloid behavior of the former.

The hydration capacity of the soaps varies with the basic radical and, in general, in the order: NH_4, K, Na, Li, Mg, Ca, Fe, Cu, Hg. It varies also with the type of fatty acid, that standing lowest in any homologous series being least hydratable. When different series are compared, the less saturated possess the lower hydration capacity.

Colloid soap jellies may be prepared from the various soaps and anhydro is solvents, like monatomic, diatomic, and triatomic alcohols, aldehydes, benzene, toluene, etc. This finding speaks against the too heavy emphasis now being placed upon purely electrical notions of stabilization in colloid systems.

Hydrophilic or lyophilic colloids are defined as dispersions, coarser than molecular, of one material in a second with the dispersed substance a solvent for the dispersion medium.

Liquid soaps may be "coagulated" by alkalies and neutral salts of various kinds even when chemical reaction between soap and added substances is impossible. The consecutive changes of setting, secondary liquefaction with progressive dehydration of the soap, and its separation in "coagulated" form are explained on the basis of union between added salt and water with production of an emulsion of salt water in soap succeeded by one of soap in salt water and ending in anhydrous soap floating upon salt water.

Chemically neutral soaps do not affect an indicator like phenolphthalein in concentrated solution. On diluting with water they turn it bright red. This means either (1) that water-in-colloid systems (analogous to normal cells and body fluids) cannot be properly analyzed by indicator methods but only colloid-in-water systems (analogous to the secretions from the body) or (2) that the former contain no ions.

From qualitative and quantitative analogy in colloid behavior between soaps and protoplasm the latter is held, in essence, to be a salt compound of various polymerized amino-acids with potassium, sodium, magnesium, calcium, etc., which materials have "dissolved" a certain amount of water. Introduction of acid, ammonia, or potassium into this compound represents poisoning by these materials at one end of such a soap-like series (as evidenced by increased hydration capacity and increased solubility in water); introduction of the heavy metals poisoning at

the other end (as evidenced by a decreased hydration capacity and the production of difficultly soluble compounds). Just as a soap anywhere in the series can, by appropriate means, be converted into any other, just so can a normal protein or living cell be "poisoned" through the addition of materials found at either extreme in the base series; or after such poisoning be changed to nearer the normal by addition of the bases in the middle series.

PATHOGENESIS DUE TO VITAMINE DEFICIENCY IN THE RAT.

By A. D. EMMETT and FLOYD P. ALLEN.

(From the Research Laboratory of Parke, Davis and Company, Detroit.)

The rats were fed on definite dietary planes which would produce normal growth except for the lack of either vitamine A or B. From these groups, animals were selected which were representative. Care was taken to see that the animals were free from infection. Control tests were made on tissues from rats fed a complete diet.

The various tissues examined histologically were: thymus, thyroid, pancreas, testes, ovaries, adrenals, liver, spleen, ileum, colon, kidneys, heart, lungs, brain, optics, lower cord, and sciatic nerves. In the absence of vitamine B, the most noticeable changes were found in the almost complete atrophy of the thymus; hypertrophy of the adrenals; passive congestion; fatty infiltration; and at times fatty degeneration in the liver and some atrophy of muscle fibers of the heart. There was more or less passive congestion in the pancreas, spleen, ileum, colon, kidneys, and lungs. In the rats which lacked vitamine A in their diet, there were no special outstanding pathological findings. In marked contrast with the lack of vitamine B the livers showed no fatty changes, the adrenals no hypertrophy, and the thymus no atrophy. The control animals proved to be normal as far as histological examination showed.

RELATION OF VITAMINES AND IODINE TO THE SIZE AND DEVELOPMENT OF THE TADPOLE.

By A. D. EMMETT, FLOYD P. ALLEN, and MARGUERITE STURTEVANT.

(*From the Research Laboratory of Parke, Davis and Company, Detroit.*)

In the light of our previous work, the temperature conditions, the exact food control, and the question of individual variation were especially borne in mind by keeping the tadpoles in an environment where the temperatures could be maintained within reasonable limits, and by keeping each tadpole in a separate vessel so that there could be no possibility of their eating each other.

The results confirmed the previous findings in regard to the size of the tadpoles; namely, that the presence of vitamines bore a direct relation to size, the water-soluble type B being more of a factor than the fat-soluble A. With respect to the development of the hind legs, the tadpoles having the vitamines in their diet did better than those without them. Vitamine B was apparently more essential than vitamine A. However, for the completion of the metamorphosis, iodine in the inorganic form or in the organic form as in the desiccated thyroid gland was essential. The relative values referred to were determined from time to time by measuring the total body length from shadow photographs, and by binocular observation every 10 to 14 days of the stage of development of the hind legs. Calculating the arithmetric mean for each group on the date observed served as the guide to the rate of advancement.

PENTOSE CONTENT OF SOME TISSUES OF MARINE ANIMALS.

By C. BERKELEY.

(*From the Marine Biological Station, Nanaimo, B. C.*)

The prevalence of complex pentosans and methylpentosans in marine algæ make it a matter of interest to examine the tissues of animals whose diet they make up for compounds of the same class.

Many observations have been made on the occurrence of pentoses in animal tissues[13] but the only systematic survey of the pentose content of various organs in one animal (the ox) is that by Grund.[14] Although guanylic acid and inosinic acid are the only compounds in which it has been shown with certainty that the pentose exists certain workers have stated that complex pentosans or methylpentosans are present in certain mollusks living on algæ.

An examination of various tissues from a variety of marine animals indicated the presence of a substance soluble in boiling water, which while it yielded furfurol was not precipitated by acetic acid from aqueous solution.

Before undertaking a further investigation of the nature of its combination it was thought important to ascertain the relative amounts of pentose in various marine animals.

For this purpose the tissues were first of all thoroughly extracted with alcohol and the air-dried residue was treated by Grund's modification of the method of Tollens and Kröbe for the determination of pentoses, the result being calculated as xylose, although it is probable that most if not all of the pentose is really present as *d*-ribose.

The following amounts of pentose were obtained: *Squalus* (dogfish) pancreas 2.28, spleen 0.67, kidney 0.65, liver 0.72, testis 0.92, heart 0.38, muscle 0.35 per cent; *Thais lamellosa* (gastropod) liver and gonads 1.15 per cent; *Evasterias troschelii* (starfish) liver 0.77 per cent.

Comparison of the results for the dogfish and for the ox as obtained by Grund show approximate correspondence, the most important difference being that there is relatively more pentose in the organs of the former except in the case of the pancreas, where the amounts are about the same in the two animals.

That the relatively high percentage in the liver (and gonads) of *Thais* may indicate that some is present in a polymerized form, is suggested by Röhmann and others for allied species.

[13] For a summary of literature see Jones, W., Nucleic acids: Their chemical properties and physiological conduct, New York, 1914, 8, 33, 34.

[14] Grund, G., *Z. physiol. Chem.*, 1902, xxxv, 111.

PENTOSAN- AND METHYLPENTOSAN-SPLITTING ENZYMES OF MACROCYSTIS PYRIFERA.

By C. BERKELEY.

(*From the Marine Biological Station, Nanaimo, B. C.*)

By the spontaneous fermentation of the giant kelp (*Macrocystis pyrifera*) in the presence of ground limestone, the kelp almost disappears in 2 to 3 weeks and the solution contains considerable quantities of the calcium salts of mono-basic fatty acids.

No free sugar occurs in the plant. The only carbohydrate constituents present in sufficient quantity to account for the acids produced are pentosan and methylpentosan complexes, the former soluble in dilute alkali and precipitated on acidification, the latter soluble in water and precipitated by alcohol.[15] Breakdown of these complexes to simple sugars must precede acid formation, but no such sugar can be detected when the fermentation takes its normal course.

If freshly cut kelp is extracted with boiling water the extract is neutral, has no reducing properties, and contains methylpentosan, but no pentosan. If the kelp has been cut into small pieces a few hours the extract is acid, reduces Fehling's solution, and contains pentosan as well as methylpentosan.

If kelp is kept for a day or so in strong alcohol and subsequently steeped in warm water containing toluene or other antiseptic, free sugar develops rapidly and both pentose and methylpentose are found in solution. After more prolonged contact with alcohol no breakdown of the polysaccharides occurs on subsequently putting the kelp into water.

By extracting fresh kelp rapidly with frequent changes of alcohol, drying at a low temperature, and grinding, a preparation can be obtained which produces free sugar very rapidly on being added to a solution of either the pentosan or methylpentosan complex. No formation of acid occurs if an antiseptic is present.

From these observations the following conclusions are drawn:

1. Kelp contains enzymes which start to break down its polysaccharide constituents as soon as it is cut.

[15] Hoagland, D. R., and Lieb, L. L., *J. Biol. Chem.*, 1915, xxiii, 287.

2. The enzymes are inactivated by prolonged contact with alcohol.

3. The production of acids sets in, in the normal course of fermentation, as soon as the sugars are set free and is probably due to the action of bacteria adherent to the kelp.

DIRECT DETERMINATION OF SODIUM IN TISSUES AS SODIUM-CESIUM-BISMUTH-NITRITE.

By R. D. BELL and E. A. DOISY.

(*From the Laboratories of Biological Chemistry, Washington University Medical School, St. Louis.*)

The insolubility of sodium-cesium-bismuth-nitrite, previously described by Ball;[16] was utilized in the development of a method for the determination of sodium in tissues.

The organic matter is oxidized by a wet ash process with a mixture of nitric and sulfuric acids. Iron must be removed from the mixture of blood salts. The solution is made faintly alkaline to methyl orange and evaporated to 2 or 3 cc. 0.5 cc. of 2 N HNO_3 and an excess of reagent are added. After standing 2 days under illuminating gas, the precipitate is filtered off on a previously weighed Gooch crucible. The reagent is removed by washing with acetone. The crucible is dried at 100° and weighed.

Since the insoluble salt is a nitrite, it may be estimated by titration with potassium permanganate. 1 molecule requires 30 atoms of oxygen for oxidation.

The precipitate contains 3.675 per cent of sodium. 1 mg. of sodium yields 27.2 mg. of the insoluble nitrite.

The reagent is made from cesium nitrate, bismuth nitrate, and potassium nitrite. Any insoluble matter which forms is dissolved by the addition of a little dilute nitric acid.

Good results were obtained in the determination of known amounts of sodium. There was good agreement between results by this method and the indirect perchlorate procedure on blood and urine samples.

[16] Ball, W. C., *J. Chem. Soc.*, 1910, xcvii, 1408.

UROCHROME EXCRETION AS INFLUENCED BY DIET.

By K. F. PELKAN.

(From the Department of Biochemistry and Pharmacology, University of California, Berkeley.)

Urochrome has long been known as a constituent of urine but its significance has never been definitely determined. Various workers have claimed its relation to the blood and bile colors and to the cellular metabolism of the organism and have denied that it was at all influenced by diet. The amount of experimental evidence submitted in favor of these claims has been small. The present work was undertaken to determine whether and to what extent the amount of urochrome excreted was dependent on the diet. Experiments carried out on a human subject resulted as follows: A low protein diet reduced the urochrome excretion up to 50 per cent, while a high protein diet raised it perceptibly. Gelatin added to a low protein diet did not affect the excretion. Colored substances were found in peptone and in amino-acid digestion mixtures which were similar in all respects to urochrome. All evidence points to the fact that urochrome is to a large extent derived from the proteins of the diet.

RELATION OF EXTRACT OF LUNG TO THE CLOTTING OF BLOOD.

By C. A. MILLS.

(From the Department of Biochemistry, University of Cincinnati, Cincinnati.)

Whereas lung extract, if injected rapidly and in sufficient amounts into animals, causes extensive intravascular coagulation and death usually in less than 1 minute, if injected in small amounts at first and then repeatedly with increasingly larger doses, causes the development of a negative coagulative phase in the blood of the animal. Such blood, withdrawn from the vessels, will not clot spontaneously for days at 5°C., putrefaction usually setting in before clotting occurs. Neither will it clot on treatment with more of the lung extract, calcium salts, thrombin, serum, CO_2 gas, or dilution with water. It differs from peptone plasma in its non-coagulability in that it contains no antithrombin or other material to inhibit the clotting of

normal blood or oxalate plasma when added to it, but instead it actually possesses a strong thromboplastic action, probably due to the presence of unchanged lung extract in it.

As much as 1,150 cc. of lung extract have been injected into an 11 kilo dog without causing intravascular clotting, whereas 3 or 4 cc. would kill a dog of this size if injected rapidly in a single dose.

The non-coagulability is not due to the disappearance of fibrinogen from the blood, for the addition of an equal volume of saturated NaCl solution to this plasma always produced a precipitate of fibrinogen. Probably the fibrinogen has been altered by the injections, since it no longer clots with thrombin.

THE RÔLE OF PHOSPHORIC ACID IN CARBOHYDRATE METABOLISM.

By CYRUS H. FISKE.

(*From the Biochemical Laboratory, Harvard Medical School, Boston.*)

The hourly inorganic phosphate output in man falls and later rises during the morning in the absence of food. After the administration of sucrose while the rate of phosphate excretion is decreasing, the fall is much more marked. The ingestion of sucrose while the rate of phosphate excretion is increasing is likewise followed by a drop in the phosphate output. In either case there is later a compensatory rise, indicating that carbohydrate combustion is accompanied by the retention of phosphate, which is later excreted at an exaggerated rate. It is suggested that this may be an indication that sugar is decomposed in the body *via* a phosphoric acid ester, as in alcoholic fermentation by yeast. The observation may account for the phosphate retention after parathyroidectomy, since the tetany caused by this operation is accompanied by rapid loss of stored carbohydrate.

UREA IN DOGFISH AND SKATE.

By C. C. BENSON.

(*From the Biological Board of Control, and the Department of Food Chemistry, University of Toronto, Toronto.*)

Determinations of urea in the muscle of dogfish and skate were made on fresh material, on material kept for varying times in cold storage and in the household refrigerator, and on material taken from cold storage and boiled.

In most cases, alcoholic solutions were used for analyses, using methods similar to those of Greene[17] and in others the ammonia was aerated directly.[18]

Fresh dogfish gave 0.5 to 0.6 per cent of nitrogen from urea, specimens from cold storage, 0.4 to 0.57 per cent of urea nitrogen, and boiled specimens showed practically no loss of urea.

Specimens of the muscle of skate, fresh or directly after taking from cold storage, gave 0.6 to 0.8 per cent of urea nitrogen but this value decreased rapidly on keeping in the refrigerator.

The quantities of urea in both forms are too small to be injurious as food, both forms keep well in cold storage, but the skate rapidly forms ammonia from its urea on being thawed.

COMPOSITION OF THE TUBERS, SKINS, AND SPROUTS OF THREE VARIETIES OF POTATOES.

By F. C. COOK.

(*From the Bureau of Chemistry, United States Department of Agriculture, Washington.*)

Data collected for 20 years by the Vermont, Maine, and New York State Experiment Stations show that an increased yield of potatoes results from spraying potato vines with Bordeaux mixture. This is true whether any *Phytophtora infestans* (late blight of the potato) is prevalent or not.

Because of the high price of copper sulfate in 1915, experiments extending over four seasons were inaugurated by the Department of Agriculture comparing the fungicidal properties of

[17] Greene, C. W., *J. Biol. Chem.*, 1919, xxxix, 435.
[18] Sumner, J. B., *J. Biol. Chem.*, 1916, xxvii, 95.

Pickering sprays (made with saturated lime water and less copper sulfate than Bordeaux) with standard Bordeaux spray.

Results with Pickering and Bordeaux sprays on two varieties of tubers at Arlington, Virginia, the past season showed an apparent increased yield of tubers and an increased percentage of solids compared with the control. Bordeaux-sprayed potatoes from several states the past season also showed increased yields and increased percentage of solids of the tubers. Green Mountain, Rural New Yorker, and Irish Cobbler tubers stored in the laboratory (average temperature 70°C.) from the time of digging in September, 1918, until sprouting had proceeded to the limit in the Spring of 1919, showed that the sprouts comprised respectively 7.2, 3.5, and 13.3 per cent of the total weight of the tubers, skins, and sprouts. A difference in the concentration or activity of the growth-promoting agencies is suggested. The analytical data for the sprouts, skins, and tubers of these and other samples show little variation in composition for the different varieties of potatoes.

Analyses of sprayed and unsprayed Green Mountain tubers, skins, and sprouts indicate that the spray did not alter the rate of growth or the composition of the sprouts.

The percentage distribution of the nitrogenous substances showed that the sprouts contained more protein, less water-soluble, about the same monoamino and amide nitrogen, and less diamino and other basic nitrogen than the tubers. The sprouts showed a selective action in withdrawing nitrogen, total ash, phosphorus, and water from the tubers in larger proportion than these constituents existed in the tubers.

GLYCOSURIA IN THREE CASES OF CHRONIC NEPHRITIS WITH EDEMA BUT WITH ONLY SLIGHT NITROGEN RETENTION.

By VICTOR C. MYERS and LUDWIG KAST.

(From the Laboratory of Pathological Chemistry and the Department of Medicine, New York Post-Graduate Medical School and Hospital, New York.)

So called "renal diabetes" has been the subject of considerable discussion since Lépine in 1895 postulated the existence of this rather interesting condition, in which the glycosuria is actually

the result of renal disease, and not due to hyperglycemia as in diabetes mellitus. About thirty cases have now been recorded in the literature. A few of these cases have shown definite evidence of renal disease aside from the glycosuria, although some would appear to be entirely free from the symptoms ordinarily associated with disease of the kidney. Renal diabetes has been compared with phlorhizin glycosuria, in which condition we have glycosuria without hyperglycemia. Some of the cases, and especially those which we wish to report, find a more direct analogy in the glycosuria of uranium nephritis. Here there is only a mild glycosuria, with a normal or nearly normal glycemia, and a constant proteinuria. Ryffel[19] and Roger[20] have each reported a case similar in some respects to our own. Our three cases (two males, aged 54 and 62, and one female, aged 10) all showed marked proteinuria and edema, but little nitrogen retention, urea nitrogen figures of 11 to 29 mg. per 100 cc. of blood. One of the cases gave a normal blood sugar, but the other two showed a mild hyperglycemia such as is ordinarily encountered in severe nephritis. In one of these the glycosuria was shown by Bailey[21] to be entirely independent of the slight hyperglycemia. The figures for the urine sugar content ranged from negative findings to slightly over 2 per cent, although when sugar was present, it most often amounted to about 0.5 per cent.

A SIMPLIFIED METHOD FOR THE ESTIMATION OF MORPHINE TOGETHER WITH DATA ON THE DISTRIBUTION OF THE ALKALOID IN ACUTE POISONING.

By SERGIUS MORGULIS and VICTOR E. LEVINE.

(From the Biochemical Laboratory, College of Medicine, Creighton University, Omaha.)

Morphine can conveniently be determined in food, tissue, or body fluid by heating with 2 per cent tartaric acid (if solid, the material should first be ground or finely minced) to convert all morphine into the soluble tartarate. The mixture is rapidly cooled, preferably on ice, to solidify the fatty material. The

[19] Ryffel, J. H., *Quart. J. Med.*, 1915–16, ix, 91.
[20] Roger, H., *Presse méd.*, 1917, xxv, 337.
[21] Bailey, C. V., *Am. J. Med. Sc.*, 1919, clvii, 221.

solid residue is removed by straining through cheese-cloth, and washed until the washings are no longer acid to litmus. The liquid, after being filtered through paper, is evaporated to a pasty consistency. The tartarate is then decomposed by the addition of an excess of solid sodium bicarbonate, which sets the alkaloid free. The evaporation is then continued to complete dryness, and the mass powdered and extracted with chloroform to remove the free morphine. The volume of the chloroform extract is noted, and the smallest quantity of the extract is found which on evaporation (in a porcelain crucible over water bath) leaves a residue which yields a definite morphine test. In this way, the relative amount of morphine in several extracts can be determined; knowing the limit of sensitivity of the reaction an approximate estimate of the amount of morphine in the original sample is possible.

The various alkaloidal tests can be applied to the residues after the evaporation of portions of the chloroform extract. The reagent employed—selenium dioxide dissolved in concentrated sulfuric acid—is very sensitive towards the opium alkaloids. It is more sensitive than many of the older reagents.

From experiments on rabbits with acute poisoning the conclusion drawn is that morphine, whether given subcutaneously or by mouth, is widely distributed throughout the animal body, finding its way into almost every tissue. The morphine is invariably found in appreciable quantities in the urine and kidney. Also large quantities may be present in the alimentary tract, liver, lungs, and brain. According to our results especially large amounts of morphine were present in the alimentary tract and excretory organs after administering the poison by mouth, while after injecting under the skin it was recovered principally from the liver, excretory organs, and also from lungs and brain. In view of these findings, it is not feasible to limit the toxicological examination for morphine to the alimentary tract *alone*. An examination of at least the kidney, urine, and liver is also indispensable.

ON THE PROTECTION AGAINST EOSIN HEMOLYSIS AFFORDED BY CERTAIN SUBSTANCES.

By C. L. A. SCHMIDT and G. F. NORMAN.

(From the Department of Biochemistry and Pharmacology, University of California, Berkeley.)

It was found that the inability of gelatin to afford any protection against the photodynamic action of eosin on red cells while marked protection is shown by blood serum, casein, edestin, and certain other proteins can be directly attributed to the lack of tyrosine and tryptophane, since the amino-acids can themselves afford marked protection. The presence of the benzene ring in a molecule does not confer protective ability while the hydroxyphenyl group appears to be one of the determining factors.

EFFECT OF ANESTHETICS ON CELL RESPIRATION.

By J. F. McCLENDON.

(From the Physiological Laboratory, University of Minnesota Medical School, Minneapolis.)

Owing to the difficulties encountered in keeping the metabolism down to the basal level in higher animals, comparative studies in metabolism are desirable. In choosing an animal for such investigations the jellyfish *Cassiopea xamachana* was decided on since the automatic activity of the nervous system may be abolished by cutting off the margin of the bell. The respiration rate is independent of the oxygen tension except at extremely low tensions of oxygen. In determining the rate of respiration, four jellyfish of large size (up to 15 cm. diameter) were deprived of manubrium and bell-margin and placed in a water-tight jar of about a liter capacity and rotated in a thermostat (kept at 30°) for 1 hour. The jar was filled up with sea water of known O_2 and CO_2 content, with the exclusion of air bubbles. The O_2 was determined by the Winkler method and the CO_2 calculated from the alkaline reserve and pH. It was found that the nervous conduction in the bell was anesthetized by 0.5 per cent ether. The jellyfish died at the end of 1 hour in 3 per cent ether, and in less than 1 hour in 4 per cent ether. The respiratory quotient

was found to be about 0.95, and since the oxygen could be determined more accurately than the CO_2 only the former will be given. The respiration was measured for 1 hour before anesthetization as a control.

	Oxygen (O_2).							
	0.5 per cent.	1 per cent.			2 per cent.		3 per cent.	4 per cent.
	cc.	cc.	cc.	cc.	cc.	cc.	cc.	cc.
Ether	2.60	2.40	1.80	2.70	1.80	2.20	1.20	1.20
Control	2.65	2.40	1.80	2.65	2.20	2.10	1.20	2.05

Within the limit of error of the apparatus the O_2 consumption was the same with or without ether, up to 4 per cent in which the jellyfish died before the end of the hour. Carbon dioxide is sometimes considered an anesthetic for marine animals. The following experiments were made by adding CO_2 to sea water of 0.0025 N alkaline reserve and determining the pH at the beginning and end of the experiment and recording the mean value. The pH for the control was always 8.15.

	Oxygen (O_2).				
	pH 6.60.	pH 6.30.	pH 5.80.	pH 5.70.	pH 5.50.
	cc.	cc.	cc.	cc.	cc.
After CO_2	2.00	1.85	1.90	0.70	0.40
Control	1.08	2.05	2.60	1.70	1.70

The addition of CO_2 reduced respiration, but the question arises whether the undissociated CO_2 molecules or the H ions were responsible for this effect. In the following experiments no CO_2 was added to the sea water, but HCl was added thus changing the character of the ionization of the "total CO_2" already present, as indicated by the change in pH. The pH of the control was 8.15.

	Oxygen (O_2).	
	pH 6.60.	pH 5.80.
	cc.	cc.
After HCl	2.02	1.30
Control	2.07	1.85

These experiments show that the CO_2 liberated from the carbonate and bicarbonate by the addition of HCl lowers the metabolism slightly. In order to show the possible effect of change in pH without increasing undissociated CO_2., acid was added to sea water and the CO_2 expelled. The pH was 5.8, and 2.9 cc. of O_2 were used as compared with a control of 2.7 cc. of O_2 in normal sea water of pH = 8.15. Since this is within the limit of error of the apparatus we presume that the H ions were not responsible for the inhibitory effect of CO_2 on respiration of the cells covering the surface of the bell of the jellyfish.

A METHOD OF INCREASING THE SHARPNESS OF THE TONE-MINIMUM IN MEASURING THE ELECTRIC CONDUCTIVITY OF CELLS.

By J. F. McCLENDON.

(From the Physiological Laboratory, University of Minnesota Medical School, Minneapolis.)

In measuring the electric conductivity of salt solutions, the sharpness of the tone-minimum is increased by shunting a condenser across the resistance box. Variable condensers sold for that purpose have a maximum capacity of about 0.003 microfarads. Our conductivity cell, when filled with distilled water, has a capacity of less than 0.003 microfarads and a condenser of that capacity may be used with it. When filled with blood, however, such a condenser is entirely inadequate, and owing to the great cost of large variable condensers a home-made apparatus was constructed to increase the sharpness of the tone-minimum. Since this title was sent in, however, we have found that a variable condenser may be built up of telephone condensers at a moderate cost. When our cell was filled with blood a capacity of 1.3 microfarads was required across the box, whereas on the addition of saponin to lake the blood a capacity of 2.2 microfarads was required.

RELATION BETWEEN CHOLESTEROL AND CHOLESTEROL ESTERS IN THE BLOOD DURING THEIR ABSORPTION.

By ARTHUR KNUDSON.

(From the Laboratory of Biological Chemistry, Union University, Medical Department, Albany Medical College, Albany.)

A series of experiments were carried out on dogs, feeding them cholesterol and cholesterol esters, respectively, to determine the relation between the two in the blood during their absorption. In a previous paper, it was shown that, during absorption of a neutral fat (olive oil), there was a marked increase of the cholesterol esters in both the plasma and the corpuscles but no significant change in the total cholesterol.

Cholesterol was determined by the Bloor method, and cholesterol esters by the Bloor and Knudson method. In all the experiments, fat was excluded from the diet. Five experiments were studied in which from 3 to 4 gm. of cholesterol were fed to dogs, and blood specimens taken every 2 hours up to 8 hours after feeding. There was found to be a marked rise in the total cholesterol both in the plasma and in the corpuscles, and in three of the five experiments the increase in the corpuscles was greater. The amount of cholesterol in the combined form as esters remained constant throughout these experiments, indicating that the cholesterol was absorbed in the free form. In a series of four experiments feeding cholesterol esters (such as palmitate, stearate, and oleate) similar increases of the total cholesterol of the plasma and corpuscles were obtained. In three of the four experiments, the increase in total cholesterol was greater in the corpuscles. There was no change in the cholesterol esters in these experiments, indicating that the cholesterol esters must have been hydrolyzed probably in the intestines before being absorbed and that they did not combine again before entering the blood.

THE FORMATION OF ACETONE BODIES FOLLOWING ETHER ANESTHESIA AND THEIR RELATION TO THE PLASMA BICARBONATE.

By JAMES J. SHORT.

(From the Laboratory of Pathological Chemistry, New York Post-Graduate Medical School and Hospital, New York.)

A study of the acetone bodies of the blood and urine was made in twelve cases with the gravimetric methods of Van Slyke and Fitz for blood and Van Slyke for urine. The blood content was but little affected during an average period of 48 minutes of ether anesthesia, but in two cases examined at intervals over a longer period there was a slight increase a few hours later and an increased output in the urine. The CO_2-combining powers in these cases increased even during increase of β-hydroxybutyric acid.

Experimental data indicate that fat in the presence of ether and ether itself may interfere with the accuracy of the method, since both may form a precipitate with the reagents and thus increase the weight of the precipitate formed from the acetone bodies. It could not be shown from analyses on postoperative blood specimens that ether directly effected much error, but through its ability to penetrate the filter it apparently carried with it some fat in solution which caused the increase. Glycerol formed a comparatively large precipitate with the reagents, oleic acid a much smaller precipitate.

It was concluded that, in the cases reported, acetone bodies were not formed promptly enough during ether anesthesia to account for the decreased plasma bicarbonate and that results of analyses for β-hydroxybutyric acid on postoperative bloods may in some instances have been too high, due to an error introduced as a result of the ether content.

PROPERTIES OF HEMOCYANIN AS AN ANTIGEN.

By C. L. A. SCHMIDT.

(From the Department of Biochemistry and Pharmacology, University of California, Berkeley.)

Hemocyanin obtained from the abalone was found to be precipitated by ammonium sulfate within the limits given by Alsberg for that obtained from *Limulus*. On repeated injections into rabbits, positive fixation and precipitin tests were obtained. The substance in moderate doses is non-toxic for guinea pigs.

ENERGY ELIMINATION AND GASEOUS EXCHANGE IN BANANAS, PINEAPPLES, AND APPLES.

By C. F. LANGWORTHY, R. D. MILNER, and H. G. BAROTT.

(From the Office of Home Economics, States Relation Service, United States Department of Agriculture, Washington.)

The changes in ripening fruit are due to enzymes normally present in the fruit and vary with the condition of the surrounding atmosphere in respect to heat, light, water vapor, and other gases. The practical improvement of storage conditions can best be secured by exact knowledge of the biological changes in the materials stored, which in turn implies an increased knowledge of the atmospheric factors just mentioned. The respiration calorimeter in this laboratory is well adapted for the study of such problems and has been used in experiments with apples, bananas, and pineapples. The bananas and pineapples were in the state known in the trade as "active ripening" while the apples were a Fall variety, recently harvested for storage for winter use.

The following table summarizes the results.

Hourly Energy Elimination and Gaseous Exchange per Kilo of Fruit Stored at Room Temperature (10–30°C.).

Fruit.	Energy.	Oxygen.	Carbon dioxide.	Water vapor.	Specific heat.
	cal.	*liters*	*gm.*	*gm.*	
Bananas	0.31	0.06	0.12	0.35	
Pineapples	0.19	0.03	0.07	0.14	
Apples	0.04				0.93

That the changes thus measured are of considerable magnitude appears from a comparison with the energy elimination of the human body at complete rest, which is estimated as 1.1 calories per kilo per hour.

INDICAN IN THE SALIVA OF A CASE OF PELLAGRA COMPLICATED WITH TUBERCULOSIS.

By M. X. SULLIVAN.

(From the Pellagra Hospital, United States Public Health Service, Spartanburg.)

The urine of many of the patients at the hospital was found to contain a high content of indican. Tests were made on the saliva to see if indican could be found therein, using the Obermayer reagent and extracting with chloroform. In Case 313 with large amounts of indican in the urine indican was found likewise in the saliva in two tests. Once the chloroform was blue with indigo blue and once red with indigo red. No other saliva showed the presence of appreciable amounts of indican. It may be noted that shortly after the discovery of indican in his saliva, Case 313 was judged to be suffering from tuberculosis also, from which disease he died about a month later. What part the tuberculosis played in the presence of indican in the saliva cannot be told.

BIOCHEMICAL STUDIES OF THE SALIVA IN PELLAGRA.

By M. X. SULLIVAN and K. K. JONES.

(From the Pellagra Hospital, United States Public Health Service, Spartanburg.)

In pellagra the condition of the mouth, and especially of the tongue, is of considerable importance in establishing a correct diagnosis. The true pellagrous tongue is vividly red and more or less swollen. The literature also speaks of salivation as a symptom of pellagra. In careful quantitative studies at the Pellagra Hospital, Spartanburg, South Carolina, some interesting data were gathered on the saliva in pellagra. It was found that, though there were cases of increased salivary flow, the salivation spoken of by the patients was often apparent rather than real

and was seemingly due to some inhibition of swallowing combined with a peculiar ropy change in the saliva or a high content of mucus which made the presence of saliva in the mouth more obvious. Occasionally also the flow was very slow, but in general it was within normal limits which vary considerably. The specific gravity of the saliva of the pellagra patients tended to be higher than that of the controls. The total solids, ash, organic matter, and mucin of the saliva was greater for the pellagrins than for the controls, but bore no relation to the mouth symptoms. The diastatic power of the saliva of pellagrins varied within the limits established by the controls. The sulfocyanate content was much less marked in the saliva of the pellagra patients than in that of normal people. The reaction of the saliva in pellagra was found to be somewhat more alkaline than that of normal saliva.

IS FIBRINOGEN FORMED IN THE LIVER?

By A. P. MATHEWS.

THE ANTIKETOGENIC ACTION OF GLUCOSE.

By P. A. SHAFFER.

A NEW QUALITATIVE AND QUANTITATIVE COLOR REACTION FOR AMINO-ACIDS.

By OTTO FOLIN and H. WU.

ON THE DETERMINATION OF BLOOD SUGAR.

By S. R. BENEDICT and ELIZABETH FRANKE.

A CONVENIENT PERMANENT UREASE PREPARATION.

By OTTO FOLIN.

ON THE LIPINS OF HUMAN CORPUS LUTEUM.

By JACOB ROSENBLOOM.

OBSERVATIONS ON THE CHEMICAL PATHOLOGY OF THE BLOOD IN PERNICIOUS ANEMIA.

By AMOS W. PETERS and A. S. RUBNITZ.

THE EFFECTS OF MODERATE HEMORRHAGE, ETC., ON SOME OF THE BLOOD ELEMENTS AND CONSTITUENTS.

By E. S. SUNDSTROEM and W. R. BLOOR.

CRYSTALLINE URIDINPHOSPHORIC ACID.

By P. A. LEVENE.

(From the Laboratories of The Rockefeller Institute for Medical Research.)

(Received for publication, November 28, 1919.)

In previous communications[1] crystalline salts of uridinphosphoric acid were described. They were the brucine salt, two ammonium salts, the lead, and the barium salt. Nearly every one has its value in the process of preparation of the free uridinphosphoric acid. The insolubility of the brucine salt is the property on which is based the separation of this from a mixture containing other nucleotides. The ammonium salt is the transition step from the brucine salt to every other salt; the lead salt serves for separation of the nucleotide in its pure state, and hence for its identification when the nucleotide is available in only small quantities. For final identification the crystalline nucleotide offers the most satisfactory material. It was prepared in the following manner.

The diammonium salt described in the previous communication was converted into the lead salt. This was suspended in water, and through the suspension a stream of hydrogen sulfide gas was passed. The filtrate from lead sulfide was freed from hydrogen sulfide by distillation under diminished pressure at room temperature. To the clear solution of the nucleotide a solution of neutral lead acetate was added to form again the lead salt. The lead salt was again treated with hydrogen sulfide as before, the resulting clear solution was concentrated under diminished pressure to a small volume, and then placed in a vacuum desiccator, where it was allowed to concentrate slowly under diminished pressure. When the solution attained the thickness of glycerol it was dissolved in hot 99.5 per cent alcohol and again placed in a vacuum desiccator and allowed to concentrate under diminished

[1] Levene, P. A., *Proc. Soc. Exp. Biol. and Med.*, 1917, xv, 21; *J. Biol. Chem.*, 1918, xxxiii, 229; 1919, xl, 395.

pressure over sulfuric acid. This operation was repeated many times and finally the thick syrup crystallized into a nearly solid, sticky mass. In order to separate the crystals from the viscous mother liquor the material was triturated with a very small quantity of hot anhydrous methyl alcohol. The crystalline material was then washed with cold methyl alcohol, and finally the substance was suspended in dry methyl alcohol. The mixture was brought to a boil and then the crystals were filtered off. The mother liquors and the wash alcohol on standing under diminished pressure over sulfuric acid gave a second crop of crystals. The substance had the melting point M. P. = 202° (corrected) with decomposition and the following composition.

0.1000 gm. of the substance gave 0.1231 gm. of CO_2 and 0.0376 gm. of H_2O.
0.2000 " " " " employed for Kjeldahl nitrogen estimation required 12.36 cc. of 0.1 N acid for neutralization.
0.3000 gm. of the substance gave 0.1020 gm. of $Mg_2P_2O_7$.

	Calculated for $C_9H_{13}N_2PO_9$.	Found.
	per cent	*per cent*
C	33.32	33.40
H	4.05	4.18
N	8.64	8.65
P	9.58	9.48

The optical rotation of the substance was

$$[\alpha]_D^{20} = \frac{+0.21 \times 100}{1 \times 2} = +10.5$$

Thus every one of the four nucleotides composing the molecule of yeast nucleic acid has now been prepared in crystalline form.

THE DETERMINATION OF SACCHARIN IN URINE.

By GEORGE S. JAMIESON.

(From the Bureau of Chemistry, U. S. Department of Agriculture.)

(Received for publication, November 25, 1919.)

The method to be described is based upon the quantitative extraction of the saccharin with ether from the acidified urine which had been previously treated with normal lead acetate and filtered. The sulfur of the extracted saccharin was determined as barium sulfate, after the fusion of the residue from the ether with sodium carbonate. The amount of saccharin present was found by multiplying the weight of the barium sulfate by 0.7844. Ether was chosen as the most suitable solvent because it had been shown by Marden[1] that saccharin could be readily extracted in a quantitative manner with ether from aqueous solutions which had been acidified with hydrochloric acid. For references upon the determination of saccharin the reader may consult the researches described by Gnadinger,[2] and Seeker and Wolf.[3]

It has been found by employing the modification of the sulfur method used for the determination of saccharin in food substances, described below, that satisfactory results can be obtained for amounts of saccharin varying from 1 mg. upwards per 100 cc. of urine. Smaller amounts of saccharin could probably be estimated with some accuracy by using larger quantities of urine for the analysis. A detailed description of the procedure will be given so that other analysts may obtain accurate results with this method.

In order to test the method, a standard solution was prepared which contained 1 mg. of saccharin per cc. The saccharin used was a high grade commercial product which had been previously analyzed in order to determine its purity. It melted at about

[1] Marden, J. W., *J. Ind. and Eng. Chem.*, 1914, vi, 315.

[2] Gnadinger, C. B., *J. Assn. Off. Agric. Chem.*, 1917, iii, 23–33.

[3] Seeker, A. F., and Wolf, M. G., *J. Assn. Off. Agric. Chem.*, 1917, iii, 38–43.

220°C. The ignition of about 0.2 gm. on a platinum foil left no perceptible residue. The amount of actual saccharin present in the sample was determined by the well known Reid method[4] and it was found to contain 98.79 per cent (average of two closely agreeing analyses). On the basis of the analyses, 2 liters of solution were prepared so that 1 cc. should contain 1 mg. of saccharin. This solution was standardized by determining the amount of saccharin present in measured volumes by the sulfur method described below. The average of two closely agreeing analyses gave the value 1 cc. = 0.00100 gm. of saccharin, which is in agreement with the results previously obtained by the Reid method. Measured quantities of the standard saccharin solution were added to 100 cc. portions of urine which was sometimes fresh and at other times 1 or 2 days old. To each solution, 10 cc. of a 20 per cent normal lead acetate solution were added and after thorough stirring the solution was allowed to stand for an hour before filtration. It was found essential to heat urine which contained albumin almost to boiling for a few minutes, and then add the lead acetate solution, otherwise unbreakable emulsions were formed when the aqueous solution was agitated with ether to extract the saccharin. After heating, the solutions were allowed to stand for 1 hour before filtration as previously described.

Since it is important to add a slight but distinct excess of lead acetate over that required for the precipitation of the compounds which if left in the solution would interfere with the extraction of the saccharin by ether, by causing the formation of emulsions, it is necessary to make some preliminary experiments to determine the proper volume of lead acetate required for each urine. It has been observed that a weak urine requires somewhat less lead acetate than a strong, highly colored urine. However, after a little experience, one can closely estimate the proper amount of lead acetate required, in the majority of cases, from the color of the urine to be examined. On the other hand, if more than a slight excess of lead acetate over the required amount was present, the precipitated lead chloride formed when the solution was strongly acidified with hydrochloric acid previous to the ether extractions was found to interfere with the complete recovery of

[4] Reid, E. E. *Am. Chem. J.*, 1899, xxi, 461.

the saccharin present. The solution was filtered by suction through a mat of paper pulp about 0.5 cm. thick. The paper pulp was prepared by stirring filter clippings with enough concentrated hydrochloric acid to moisten it until it was thoroughly disintegrated. Then about 15 volumes of water were added and, after shaking, the mixture was ready for use. When the proper thickness of mat had been obtained in a 6 cm. funnel, it was washed three times with about 50 cc. of water each time to remove the acid. When the lead precipitate was in the funnel and most of the solution had filtered through, it was washed four times, using about 10 cc. of water each time. During the first part of the filtration, a gentle suction was used, but when the precipitate was ready for washing, the full suction was used. The suction was continued until no more filtrate was obtained even after the precipitate had been firmly pressed to a hard mass with a spatula. It should be added that the funnel and precipitate were washed again with a small quantity of water after the precipitate had cracked just before pressing it down with the spatula. It was found that the filtration, including the washing and pressing of the precipitate, usually required about 1 hour. It is most important that every piece of apparatus used in the analysis be rinsed absolutely free from sulfates and furthermore the analysis must be made in a room where sulfuric acid is not volatilized. The filtrate was transferred to a suitably sized separatory funnel and the filtering flask was rinsed twice with about 5 cc. of water. Then 15 cc. of concentrated hydrochloric acid were added and, after mixing, 55 cc. of ether were added. The contents of the funnel were shaken for 2 minutes. After the layers had separated for about 10 minutes, the aqueous solution was drawn into a clean beaker. The ether was rotated in order to wash the sides of the funnel as free as possible from water. When the water had settled to the bottom of the funnel, it was tapped off. The ether was poured into a 600 cc. beaker and evaporated by directing a gentle current of air on the surface. The aqueous solution was returned to the funnel and extracted again with 50 cc. of ether in precisely the same manner as before. The second ether extract was added to the beaker containing the first extract and a third extraction using the same volume of ether was made. When the combined ether extracts had been evaporated as completely as

possible with the air current, the remaining moisture was removed by heating the beaker on a steam bath. Care was taken not to heat so strongly as to cause the solution to boil. It is very important not to allow a single drop of aqueous solution to be carried along when transferring the ether to the beaker from the separatory funnel. The residue from the ether was allowed to cool to room temperature and treated with about 12 cc. of absolute ether by pouring it around the upper edge of the beaker so as to wash down the saccharin adhering to the sides. The ether was allowed to remain on the residue for 5 minutes; then it was transferred to a 30 cc. nickel crucible. While the ether was being evaporated by a gentle current of air directed on the surface of the liquid, the residue was extracted again with 12 cc. of absolute ether. This ether extract was added to the crucible. A third portion of about 15 cc. of absolute ether was added to the residue which was detached from the sides and bottom of the beaker by rubbing with a stout glass rod. A rubber-tipped rod must not be employed. This extract was filtered to remove the suspended sediment through a 5 cm. filter into the crucible. The beaker was rinsed and the residue was stirred with another 15 cc. portion of ether which was filtered and added to the crucible. Then the filter was washed by adding about 5 cc. more of ether around the upper edge. When the ether had evaporated, about 2 gm. of anhydrous sodium carbonate were added. The sodium carbonate was spread, by means of a small spatula, up the sides of the crucible as far as the residue extended. The nickel crucible was placed in a porcelain crucible and heated for 10 minutes with a low flame. Then the flame was gradually increased to its full capacity. At this point, the porcelain crucible was removed and the nickel crucible was heated directly for 5 minutes at the highest temperature of the burner. When the crucible was cold, 20 cc. of water were added and, after standing for about 10 minutes, the contents of the crucible were heated almost to boiling. The solution was filtered through a 9 cm. washed filter and the crucible was rinsed several times with warm water. After washing the filter four times with water, the beaker containing the filtrate was covered and the solution was acidified with 5 cc. of hydrochloric acid. The solution was heated for 5 minutes on the steam bath before adding 10 cc. of a 10 per cent solution of barium chloride to

precipitate the sulfate. When the solution had been heated for about 3 hours, it was allowed to cool to about 50°C. It was filtered on a carefully prepared Gooch crucible and washed with 60 to 75 cc. of water. In filtering small amounts of barium sulfate, it was found best to use a very gentle suction until after the final washing had ceased running through. At this point the suction was increased very gradually until the maximum was reached. The crucible containing the precipitate was placed in a porcelain crucible and heated for a short time with a small flame. Then it was heated with the full flame for 5 minutes. When the crucible

TABLE I.

No.	Saccharin taken with 100 cc. urine.	Barium sulfate found.	Saccharin calculated.	Error.
	gm.	*gm.*	*gm.*	*gm.*
1	0.0120	0.0149	0.0117	−0.0003
2	0.0120	0.0153	0.0120	0.0000
3	0.0080	0.0099	0.0078	−0.0002
4	0.0080	0.0102	0.0080	0.0000
5	0.0005	0.0007	0.0005	0.0000
6	0.0051	0.0064	0.0052	+0.0001
7	0.0040	0.0050	0.0039	−0.0001
8	0.0030	0.0040	0.0031	+0.0001
9	0.0010	0.0015	0.0012	+0.0002
10	0.0020	0.0027	0.0021	+0.0001
11	0.0020	0.0032	0.0024	+0.0004
12	0.0030	0.0040	0.0031	+0.0001
13	0.0050	0.0060	0.0047	−0.0003
14	0.0050	0.0062	0.0050	0.0000
15	0.0100	0.0126	0.0099	−0.0001

had cooled for 20 minutes it was weighed. The weight of barium sulfate obtained after deducting the blank correction described below was multiplied by 0.7844 to obtain the weight of saccharin which it represented. A blank analysis for sulfate was made using all the reagents in the same amounts as in the actual experiments and less than 0.1 mg. of barium sulfate was obtained. Several blank analyses were made at various times carrying out the entire process with 100 cc. portions of urine as described above. The average of the blank analyses amounted to 0.7 mg. of barium sulfate which has been deducted from the weights of barium sulfate given in Table I.

From the table of analyses it will be observed that very satisfactory results were obtained. In order to obtain good results, it is most essential that the directions given above should be executed with the greatest care possible. Furthermore, before attempting to apply this method a sufficient number of blank analyses should be made, if possible, upon urine of the subject to be experimented upon, so that the proper correction can be applied in connection with the determination of saccharin.

SUMMARY.

Urine is treated with a solution of lead acetate and filtered. After acidifying with hydrochloric acid, the saccharin is extracted by ether, the ether removed by evaporation, and the saccharin extracted from the residue with ether. The ether is removed and the resulting residue is fused with sodium carbonate and the sulfur determined as barium sulfate. The amount of saccharin is calculated from the sulfur found after making a correction for the blank. The experimental results obtained by the analysis of urines, to which known amounts of saccharin had been added, show that the method is capable of giving satisfactory results providing that the directions are followed exactly as described in every detail.

A THERMOREGULATOR WITH THE CHARACTERISTICS OF THE BECKMANN THERMOMETER.

By R. B. HARVEY.

(From the Bureau of Plant Industry, United States Department of Agriculture, Washington.)

PLATE 1.

(Received for publication, November 17, 1919.)

In working at low temperatures in a constant temperature chamber it was found desirable to reduce the heat capacity of the thermoregulator as much as possible in order to give quick response to small changes in temperature of the air. It was found advantageous also to have the thermoregulator show the same lag effects as the standard measuring instrument, the Beckmann thermometer.

The instrument here shown (Plate 1) can be set easily at any temperature desired between -20 and $+250°C$. Approximate settings can be made by warming or cooling the mercury bulb until the upper level of the column reaches the desired point on the setting scale. For making more accurate settings a slight blow will separate a series of small globules of mercury from either portion after the mercury is divided approximately by means of the scale. As many of these droplets as are desired can be added to the contents of the bulb by turning the instrument upside down. The capillary is sufficiently large to allow the mercury in the bulb to flow through it easily when the column is broken by a slight blow with a pencil. The instrument is thus more easily set than the ordinary Beckmann thermometer.

The lower platinum contact should be made in an enlargement of the capillary to prevent sticking of the mercury at that point. The upper contact is placed at the end of the capillary so that small temperature changes will make or break the circuit. The mercury column is of sufficient diameter to carry the current required to operate a 150 ohm relay using 2 or 4 volts. The heating circuit should be broken by a relay.

When run on test in the air bath with a standard Beckmann thermometer the air temperature was regulated within ±0.004°C. when stirred vigorously. The accuracy of regulation of the air temperature varies greatly with the heat capacity of the source of heat and the rate of stirring.

(Harvey: Thermoregulator.)

THE DETERMINATION OF HYDROGEN ION CONCENTRATION.

By JOHN W. M. BUNKER.

(*From the Department of Bacteriology, Digestive Ferments Company, Detroit.*)

(Received for publication, November 12, 1919.)

The reasons for the measurement of reaction of culture media and biologic fluids in terms of pH are well covered by numerous publications upon the subject. The art of making the determination is still susceptible of great variations in method. Certain principles concerning the technique of electrolytic determinations are important. The electrode itself should be as simple in construction as possible, in order that it may be easily cleaned and easily replaced. The measurement of small quantities of test liquid should be possible. Easy attainment of equilibrium in the solution should be expected. Those parts of the apparatus which come in contact with test liquids should be easily subjected to sterilization.

An electrode and vessel are here described which fulfil some of these requirements. In its design, ideas have been borrowed freely from published work and through personal observations. It is a modification of the bubbling electrode, used in a closed vessel.

The tube used to conduct the hydrogen gas to the platinum wire of the hydrogen electrode is the essential part of this apparatus. It is indicated by E in Fig. 1. This is a piece of glass tubing of $\frac{5}{16}$ inch internal bore with a side arm of small diameter joined to it at approximately 1 inch from the top. The $\frac{5}{16}$ inch tubing is about 5 inches long over all. In its preparation it is sealed at the bottom and then heated on one lower "corner," and a bubble at this point is blown and later broken with a file. The edges of the lip which remain are fire-polished, and then the end of the tube is heated and the round hole thus obtained is contracted to an approximately oval shape.

The hydrogen electrode itself, B, is made from a piece of $\frac{1}{8}$ inch tubing with a short piece of platinum wire sealed in at the bottom, projecting outside about $\frac{1}{8}$ inch, and inside far enough to make contact with mercury C, with which the lower part of this tube is filled. A small rubber stopper A is fitted tightly around this tube at about 1 inch from the top. The entire electrode is about 6 inches long. In assembling the apparatus, the electrode tube B

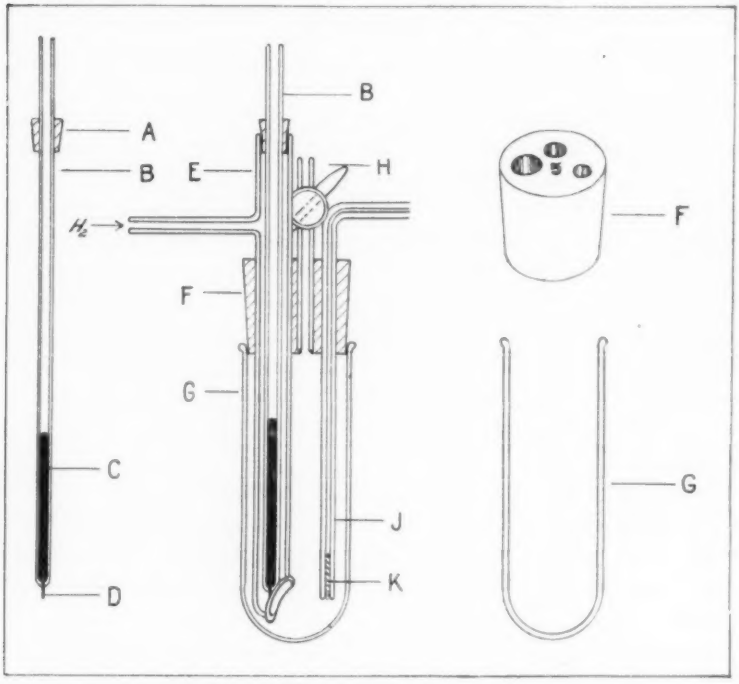


FIG. 1. Diagram of hydrogen electrode.

with its stopper A is inserted in tube E and pushed down tightly, and so adjusted that the platinum wire D is opposite the orifice in the lower end of the tube E. The exact point of this must be determined by trial, as must the size and shape of the orifice. When the assembled electrode is dipped in a liquid, and gas is bubbled through it in the direction indicated, there should form a large bubble at the base of the tube E which is held together

by the walls of the tube until it has travelled far enough for the level of the liquid within the tube to have reached a point below the platinum wire, thus exposing the wire to hydrogen gas. The bubble is pushed out of the tube and, as it leaves, the liquid rushes in behind it up into the tube, and should again bathe the platinum needle in liquid. This gives the effect of the rocking electrode of Clark,[1] in which the platinum electrode itself is bathed alternately in liquid and in gas. If the orifice is too wide, the bubble will be pushed out before it has travelled far enough to clear the platinum needle, and in that case the tube E must be dried, reheated in the flame, and the opening constricted.

The electrode vessel G which we call a pH tube, is an ordinary test-tube of 1 inch internal diameter, and approximately 3 inches in length. The exact dimensions are not important. We have a supply of these tubes on hand, carefully washed and dried properly, and stored away ready for determinations. When a series of determinations of pH is to be made, the proper number of tubes is selected, each is given its proper liquid, and is set up in a rack ready to use. One by one each may then be pushed up under the hydrogen electrode against the rubber stopper F and, as soon as the determination is read, slipped out to make way for the next.

The rubber stopper F is a No. 5 stopper with three holes made by cork borers to receive tightly the tube E and the outlet tube H with its stop-cock, and to receive not so tightly, the potassium chloride arm J, which is made of small capillary tubing. The rubber stopper is slipped up over the hydrogen electrode tube E and fastened to it with cement or shellac. The outlet tube H which carries a stop-cock is inserted into the rubber stopper, and also cemented in place. The stopper remains on the tube E at all times. The capillary tube J plugged at the end with a bit of cotton wicking K makes the connection between the saturated potassium chloride of the calomel half-cell and the test liquid. The tube J slips up and down in the stopper and should fit as tightly as possible, still allowing the necessary motion. The apparatus is assembled as in Fig. 1. Hydrogen gas is bubbled through the inlet tube for approximately 30 seconds at a rapid

[1]Clark, W. M., *J. Biol. Chem.*, 1915, xxiii, 475.

rate, with the outlet tube H open and the potassium chloride arm J lifted out of the test liquid. Then the stream of hydrogen is slowed down to such a rate that the bubble travels in the lower end of the tube E once every 2 seconds. This is maintained for about 1 minute. The relation of the parts, the volume of liquid, and the size of the bubble of hydrogen liberated are such that adequate stirring of the test liquid is obtained by the bubbling, and it is not necessary to shake or rock the apparatus. At the end of the bubbling the hydrogen is shut off, by an external stop-cock, just after a bubble of gas has been liberated from the tube E and the liquid has assumed a level in the tube above the platinum needle D. The stop-cock in the outlet tube is immediately closed, thus insuring an atmosphere of hydrogen gas over the test liquid in a closed vessel. The potassium chloride arm J is then lowered so that contact is made, and reading is taken by whatever electrometric apparatus is available.

If it is desirable to avoid all chance of contact-potential error, the wicking K in the potassium chloride arm J may be dispensed with, and, after the arm J is lowered to the bottom of the pH tube E, potassium chloride may be run in under the test solution in order to make a wide zone of contact between the potassium chloride and the test solution, the arm J, of course, being lowered to make contact with the volume of potassium chloride in the bottom of the tube G, and the stop-cock outside of the apparatus closed to prevent further inflow of potassium chloride. There will always be enough potassium chloride between the stop-cock and its jacket to transmit the necessary E. M. F. In the writer's opinion the error is less and the convenience greater if the wicking is retained in the potassium chloride arm, and the outside stop-cock is left open in order to insure plenty of electrolyte along the whole course which the current must travel.

The apparatus is designed to meet the requirements of quick, accurate determinations in large numbers, and has been in use satisfactorily for a long period of time. A number of electrode parts B may be made from the same piece of platinum wire and, when treated in the same way as to blacking, may be substituted in the apparatus when necessary without loss of accuracy. All electrodes must of course be tested as to blacking against a standard acetate or other known buffer solution.

ON THE IDENTIFICATION OF CITRIC ACID IN THE TOMATO.

By R. E. KREMERS and J. A. HALL.

(*From the Phytochemical Laboratory of Edward Kremers, University of Wisconsin, Madison.*)

(Received for publication, November 11, 1919.)

In spite of the large amount of work which has been done on the chemistry of the tomato, Hansen,[1] working in this laboratory a few years ago, was the first to isolate citric acid in crystal form from the juice of the tomato and to make an elementary analysis of his material. With more material available, it was desired to repeat Hansen's experiments. The recent work of Reid[2] and his colaborers suggested the application of derivatives of citric acid to its identification.

Citric Acid Triphenacyl Ester.—In order to familiarize ourselves with the procedure, the reaction was applied to U. S. P. sodium citrate. Instead of using the phenacyl bromide in slight excess, as Reid suggests, our experience seems to indicate that a slight excess of sodium citrate is preferable. The record of a typical experiment is as follows: 3.263 gm. of sodium citrate were dissolved in 27.5 cc. of water and 5.4114 gm. of phenacyl bromide and 55 cc. of 95 per cent alcohol were added. Alcohol has the property of preciptating an aqueous solution of Na citrate. On continued refluxing with frequent agitation the aqueous layer gradually disappears. The attainment of homogeneity is a good indication that the reaction is nearly complete. Refluxing on a boiling water bath was continued for 4 hours. The reaction mixture was poured into a crystallizing dish, set upon the water bath after the

[1] Unpublished results.

[2] Reid, E. E., *J. Am. Chem. Soc.*, 1917, xxxix, 124. Lyman, J. A., and Reid, E. E., *J. Am. Chem. Soc.*, 1917, xxxix, 701. Lyons, E., and Reid, E. E., *J. Am. Chem. Soc.*, 1917, xxxix, 1727. Rather, J. B., and Reid, E. E., *J. Am. Chem. Soc.*, 1919, xli, 75.

flame had been extinguished, and allowed to stand. If in a day or two the precipitate has not crystallized, it is redissolved in hot 95 per cent alcohol, from which it is usually obtained crystalline. Melting point 105°. Reid found 104°.

Unfortunately Reid gives no analytical data for his compounds. The following results were obtained with our material.

Combustion.—(No. 1.) 0.2159 gm. of ester gave 0.0975 gm. of H_2O and 0.5243 gm. of CO_2.

(No. 2.) 0.2452 gm. of ester gave 0.1133 gm. of H_2O and 0.5907 gm. of CO_2.

	Carbon. *per cent*	Hydrogen. *per cent*	Oxygen. *per cent*
No. 1	66.23	5.00	28.77
" 2	65.70	5.14	29.16
Theoretical	65.93	4.76	29.31

Saponification.—A quantitative saponification was undertaken several times; each time the results were too high for a tri-ester and no definite end-point could be located. Even cold N/2 NaOH gave too high results. According to Beilstein, phenacyl alcohol is readily decomposed even by dilute alkali.

Although Reid had obtained practically negative results in his attempts to prepare mono-*p*-nitrobenzyl esters of di-basic acids, it seemed worth while to try at least a few orienting experiments along the same line before proceeding with the acids from the tomato. Accordingly di- and monosodium citrate were prepared according to the directions of Heldt.[3]

The phenacyl esters were prepared in the same manner as the tri-ester; *i.e.*, the Na salt was slightly in excess of the theoretical quantity. The esters were crystallized as before.

	Di-ester. °C.	Mono-ester. °C.
M. P. of ester	104–105	104.5
M. P. mixed with tri-ester	105	104.5

Therefore the triphenacyl ester separates in each case. Perhaps the simplest explanation, in the absence of more experimental data, is that Heldt's mono- and disodium citrates are not truly named, but are merely mixtures of trisodium citrate and citric

[3] Heldt, W., *Ann. Chem.*, 1843, xlvii, 157.

acid having the analytical composition indicated by their names. The method of preparation and the fact that Heldt prepared no corresponding salts by double decomposition with other metals rather support this supposition.

Application to the Tomato.

Material.—The material was obtained by saturating strained tomato pulp[4] with calcium hydroxide; the Ca citrate is thereupon precipitated by boiling. That the material thus prepared is not nearly pure Ca citrate is shown by the analysis for Ca.

Found 15.6 per cent. Ca citrate requires 24.1 per cent.

The impure Ca salt did not yield crystals when brought into reaction with phenacyl bromide. Its purification was undertaken as follows after various trials:

The impure Ca salt was converted into the Na salt by boiling with Na_2CO_3. Ca citrate was reprecipitated by adding NH_4Cl and $CaCl_2$ in excess to the solution of the Na salt, boiling and filtering while hot. The Ca salt dissolves in 50 per cent acetic acid on continuous stirring, from which solution the Pb salt is precipitated by basic Pb acetate. The Pb salt was decomposed by H_2S, the citric acid solution filtered from the Pb sulfide, concentrated *in vacuo*, and crystallized in a vacuum desiccator. The triphenacyl ester was then prepared as follows:

0.7382 gm. of citric acid dissolved in 9 cc. of water was neutralized with 0.5389 gm. of Na_2CO_3. 1.61 gm. of phenacyl bromide dissolved in 18 cc. of alcohol were added and the mixture was refluxed for 3 hours. The reaction mixture was crystallized; yield 0.52 gm., melting point 104–105°. Mixed with ester from U. S. P. sodium citrate, melting point was 104–105°.

Therefore the presence of citric acid in the tomato juice has been shown by means of its triphenacyl ester.

[4] For the material at our disposal we are much indebted to the Tomato Products Company of Paoli, Ind.

THE STRUCTURE OF YEAST NUCLEIC ACID.

V. AMMONIA HYDROLYSIS.

By P. A. LEVENE.

(From the Laboratories of The Rockefeller Institute for Medical Research.)

(Received for publication, December 1, 1919.)

In the preceding publication of this series[1] the statement was made that on mild hydrolysis with 5 per cent ammonia at a temperature of 100°C. yeast nucleic acid is broken up into four mononucleotides. The publication contained a report of the isolation of only three of these. At the time when that publication went to press, the fourth nucleotide had been isolated in the form of the brucine salt. However, we have learned that for the identification of a nucleotide one cannot depend on the analysis of only the brucine salt, when this salt is obtained from a mixture of brucine salts of several nucleotides.

The present publication contains a report on the isolation of the fourth nucleotide, the crystalline cytidinphosphoric acid. It also presents an example showing that not only the brucine salts, but also the free nucleotides are capable of forming mixed crystals, which may furnish analytical data very nearly approaching those required by polynucleotides. A crystalline substance was isolated, which, on the basis of its elementary analysis, could easily have been taken for an adenosin-uridin dinucleotide. On recrystallization from dilute alcohol, the substance was fractionated into cytidinphosphoric and adenosinphosphoric acids. Thus it is evident that the danger of error is extremely great if one assumes the existence of di- or trinucleotides on the basis of the results of elementary analysis.

EXPERIMENTAL.

The material for the present work was the brucine salts of the adenine fraction. It was stated in the preceding communication that these brucine salts were recrystallized nine times out of

[1] Levene, P. A., *J. Biol. Chem.*, 1919, xl, 415.

35 per cent alcohol. The final product consisted of the brucine salt of uridinphosphoric in a practically pure state. This was demonstrated by the fact that, when the brucine salt was converted into the ammonium salt, this without further purification was in a crystalline state and had an elementary composition required by the theory of that salt.

The first three mother liquors combined and concentrated yielded a brucine salt containing 10 per cent nitrogen, which served for the isolation of adenosinphosphoric acid described in the preceding publication.

The mother liquors from the fourth to the ninth (inclusive) recrystallizations served as starting material for the isolation of cytidinphosphoric acid. The crude brucine salts obtained on concentration of the mother liquors were first fractionated by means of methyl alcohol. For this purpose the brucine salts were suspended in methyl alcohol, boiled for 15 minutes on boiling water bath, and filtered while hot. There were obtained an insoluble part A and the mother liquor, which on cooling deposited a sediment B; the mother liquor from B on concentration under diminished pressure gave a third precipitate C. The last was too small for further work. Fraction A was recrystallized three times out of 35 per cent alcohol. The brucine salt had a nitrogen content N = 7.71 per cent. It was converted into the ammonium salt, and this into the lead salt. The latter was suspended in water. Through the suspension hydrogen sulfide gas was passed and the filtrate from lead sulfide was concentrated to small volume under diminished pressure, finally placed in vacuum desiccator over sulfuric acid, and allowed to crystallize under diminished pressure. When the solution was concentrated to the consistency of a syrup, there began to form a crystalline deposit consisting of long needles. The mother liquor was very viscous and the crystals were freed from it by repeated washing with hot methyl alcohol. Apparently the mother liquor contained some uridinphosphoric, which is extremely soluble in water.

The crystalline substance was analyzed without recrystallization. It had a melting point M.P. = 225°C. (corrected) with decomposition. The analysis of the substance was as follows.

0.1106 gm. of the substance employed for Kjeldahl nitrogen estimation required 10.34 cc. of 0.1 N acid.

	Calculated for $C_9H_{14}N_3PO_8$. per cent	Found. per cent
N	13.00	13.09

The optical rotation of the substance was as follows.

$$[\alpha]_D^{20} = \frac{+1.05 \times 100}{1 \times 2} = +52.5$$

The rotation of the cytidinphosphoric acid isolated by Thannhauser was $[\alpha]_D^{20} = +23.3$, and the value calculated from the barium salt described by the writer was also $[\alpha]_D^{20} = +23$. The reason for this discrepancy will have to be established. The two latter preparations were obtained on acid hydrolysis.

Fraction B was recrystallized four times out of 35 per cent alcohol. The resulting substance was converted into the ammonium salt, which in its turn was converted into the lead salt, and this was freed from lead and thus a solution of free nucleotides was obtained. This was concentrated under diminished pressure and the solution allowed to crystallize in the open air. Crystallization began rapidly and was completed after 3 days. The crystal form differed from that of the pure nucleotides. The latter appear in form of long fine needles, while these had the appearance of heavy prisms. The substance was recrystallized once out of hot water and then had the following composition.

0.1170 gm. of the substance gave 0.1453 gm. of CO_2 and 0.0419 gm. of H_2O.

0.1930 gm. of the substance employed for Kjeldahl nitrogen estimation required 20.60 cc. of 0.1 N acid for neutralization.

0.2845 gm. of the substance gave 0.0954 gm. of $Mg_2P_2O_7$.

	Calculated for $C_{18}H_{28}N_7O_{18}P_2$. per cent	Found. per cent
C	34.90	33.86
H	3.86	4.12
N	15.01	14.94
P	9.50	9.19

The optical rotation of this substance was

$$[\alpha]_D^{20} = \frac{+0.40 \times 100}{1 \times 2} = +20.0$$

Thus the substance could easily be taken for a dinucleotide. It was separated into two fractions in the following manner: 4.8 gm. of the substance were dissolved in 150 cc. of hot water, and to this solution 300 cc. of 99.5 per cent alcohol were added gradually. On cooling a crystalline deposit began to form. After 1 hour this was removed by filtration. The yield of the air-dry material was 2.8 gm. (Fraction A). In the mother liquor on concentration under diminished pressure a deposit formed consisting of long needles (Fraction B).

Fraction A_1 analyzed as follows.

0.1006 gm. of the substance gave 0.1216 gm. of CO_2 and 0.040 gm. of H_2O.
0.2000 " " " " employed for Kjeldahl nitrogen estimation required 18.92 cc. of 0.1 N acid.
0.2940 gm. of the substance gave 0.0982 gm. of $Mg_2P_2O_7$.

The optical rotation of the substance was

$$[\alpha]_D^{20} = \frac{+0.80 \times 100}{1 \times 2} = +40.0$$

Once recrystallized out of water the substance analyzed as follows.

0.0986 gm. of the substance gave 0.1206 gm. of CO_2 and 0.041 gm. of H_2O.
0.1973 gm. of the substance employed for Kjeldahl nitrogen estimation required for neutralization 18.33 cc. of 0.1 N acid.

The optical rotation of the substance was

$$[\alpha]_D^{20} = \frac{+0.86 \times 100}{1 \times 2} = +43$$

	Calculated for $C_5H_{14}N_2PO_8$.	Found.	
	per cent	Sample A_1. *per cent*	Sample A_2. *per cent*
C	33.42	33.57	33.35
H	4.37	4.44	4.64
N	13.00	13.51	13.01
P	9.61	9.30	

Fraction B_1 analyzed as follows.

0.2000 gm. of the substance employed for Kjeldahl nitrogen estimation required for neutralization 26.24 cc. of 0.1 N acid.

	Calculated for $C_{10}H_{14}N_4PO_7$. *per cent*	Found. *per cent*
N	20.17	18.44

The optical rotation of the substance was

$$[\alpha]_D^{20} = \frac{-0.78 \times 100}{1 \times 2} = -39.0$$

QUANTITATIVE ESTIMATION OF INDOLE IN BIOLOGICAL MEDIA.*

By HARPER F. ZOLLER.

(From the Research Laboratories of the Dairy Division, Bureau of Animal Industry, United States Department of Agriculture, Washington.)

(Received for publication, November 25, 1919.)

The demand for a rapid and yet delicate method for quantitatively estimating indole arose from a study of its progressive formation in certain bacterial cultures in this laboratory. It is rather remarkable that few methods have been proposed for determining this important product of the metabolism of certain microorganisms. Among those deserving attention are the suggestions of Herter and Foster (1), for the successful application of which we are indebted to Gorter and de Graff (2), and more especially to Bergeim (3); also the application of the nitroso reaction by von Moraczevskii (4) and by Nonnotte and Demanche (5). Both these methods depend upon the use of very sensitive color reactions.

The use of the color reaction of Ehrlich (*p*-dimethylaminobenzaldehyde) was considered but dismissed after a few trials for several reasons: (*a*) it reacts readily with phenolic compounds; (*b*) it is extremely slow in attaining equilibrium as regards its full deep color (6); (*c*) it is a difficult compound to obtain, and was expensive at the time of the investigation; and (*d*) the reagent deteriorates rapidly.

The method as devised by Bergeim, employing the very sensitive color reaction of Herter and Foster (beta-naphtha-quinone-sodium-monosulfonate), is fairly dependable and sensitive, but is objectionable in several respects. In the first place the reaction cannot be used in the presence of phenols or ammonia, which necessitates the separation of the indole from these substances by distillation. Solutions of beta-naphtha-quinone-sodium-mono-

* Published with the permission of the Secretary of Agriculture.

sulfonate deteriorate rapidly, the compound being subject to ready oxidation. At present it is difficult to obtain the reagent upon the open market, although this objection is hardly valid, since the compound could be produced if the demand were prevalent. A more serious objection, and one common to all methods involving the use of the colorimeter, is the question of the expense of the instrument. Many laboratories do not possess a standard colorimeter.

As proposed, the method of Bergeim requires the cumbersome and laborious process of steam distillation for the separation of the indole from the fecal suspensions. Since many substances seem to interfere with the accuracy of the determination it is necessary to repeat the steam distillation process, in the first instance using an alkaline medium and afterwards an acid one. Bergeim points out the use of Folin and Bell's exchange silicate (*permutit*) (7) for the absorption of ammonia, and his results seem to show that it is possible by its use to evade one of the distillations, although the employment of permutit following the first steam distillation requires considerable manipulation. In my own experience it has been found possible to remove small quantities of several of the alkylamines with exchange silicate, notably monomethyl- and monoethyl- and dimethyl-amine.

In 1908 Nonnotte and Demanche (5) published a method which proffered the possibilities of being developed into one possessing great simplicity as well as accuracy. They made use of the familiar nitrite color reaction of Baeyer (8) and Nencki (9), which involves the addition of a dilute nitrite solution to an aqueous solution of indole, and following this with a few drops of hydrochloric, sulfuric, or nitric acid. Nonnotte and Demanche specify ten drops of 1 per cent nitrite and four drops of concentrated sulfuric acid to 10 cc. of the centrifuged medium. The intensity of the color, after a given time limit, is compared to a series of standard tubes containing known quantities of indole treated as above.

Hopkins and Cole in 1903 (10) and Herter (11) in 1908 made extensive study of the chromogen of the "urorosein" urine reaction and discovered that indoleacetic acid as well as indole could be responsible for the red color produced when urines containing nitrites were treated with HCl or H_2SO_4. Herter also found that

p-dimethyl-amino-benzaldehyde reacted with indoleacetic acid to give a coloration similar to that with indole. With this in mind it is obvious that Nonnotte and Demanche's use of the nitroso reaction in cultures, freshly centrifuged to relieve turbidity, is fraught with grave danger, since indoleacetic acid (skatole carbonic acid, Salkowski (12)) is reported to have been isolated from cultures of *Bacillus coli* by Hopkins and Cole (10) and putrefactive mixtures by Salkowski (12). Homer (6), in a recent and very exhaustive study of the chromogen of the urorosein and allied reactions, has more clearly identified the precursors of these color changes. For most purposes it should be conceded that, in order to render even the detection of indole in bacterial cultures and other media at all sensitive and reliable, it is necessary to apply the test to the distillate after proper distillation. The limitations for a proper distillation formed the objective of a separate study and the results are reported in another paper (13).

Another fact which was evidently lost sight of by Nonnotte and Demanche is the solubility of nitroso-indole in weakly acid solutions. When a solution, containing a concentration of indole greater than 0.15 mg. per 10.0 cc. of solution, is treated with nitrite and acid the red nitroso-indole compound supersaturates the solution and gradually settles on the side of the container. The discovery that the sensitivity of the nitroso reaction with indole could be increased more than threefold by resorting to a few cc. of either isoamyl (primary) or isobutyl (primary) alcohol at once suggested the value of the test for detection. The alcohol completely extracts the nitroso-indole and rises to the surface, thereby concentrating to a small area the full amount of color. This discovery, together with the result that these solutions of nitroso-indole possess great permanence over long periods of time, led us to believe that the method of Nonnotte and Demanche could be so improved as to furnish one of reliable application.

Separation of Indole from Complex Mixtures.

Technique.—As reported in another study (13) it was found that the method of steam distillation could be abandoned for the more available one of direct distillation, provided recognition was given to the tendency of indole to volatilize more readily from solutions

made alkaline above a certain hydrogen ion concentration and within the limit of no hydrolytic action. The particular range of hydrogen ion concentration which gave complete volatilization of the indole from the distilling flask when 75 per cent of the contents had been driven over, and which showed no indication of loss of indole through destructive action, was from pH 8.5 to 10.5; the highest alkalinity studied was pH 10.5. The point arbitrarily chosen to which all solutions should be adjusted before distilling is pH 9.2. This point was taken in the first place because of the ease of adjusting the reaction of the solution to this intensity of acidity. At pH 9.2 phenol- or cresolphthalein[1] are both at their medium of intensity of clear red-violet color, while thymolphthalein fails to show a visible blue color (the first appearance of color with thymolphthalein is between pH 9.3 to 9.4). In the second place the degree of foaming increases with protein solutions as the alkalinity increases, while at the point chosen very little trouble was experienced. Furthermore, higher alkalinities tend toward harsh hydrolysis of protein material and liberate quantities of ammonia. While small amounts of ammonia were not found to hamper the delicacy of the test in any way, large amounts may just as well be avoided.

The frothing in alkaline solutions may be checked in a large measure by the addition of various compounds, but in the case of my own experience a little care exercised during the distillation was all that was needed. One precaution that was uniformly taken was to seal one end of a glass tube about 6 to 8 mm. in diameter and insert it into the distilling flask with the open end on the bottom of the flask beneath the surface of the liquid and the opposite end resting against the neck of the flask so that it would remain in this position throughout the distillation. A constant flow of vapor is maintained by this form of ebullator and the tendency to foam is diminished. Many of the high boiling point compounds used to reduce foaming are solvents for indole and thereby tend to concentrate it in different regions within the distilling flask. Phenylether as proposed by Mitchell

[1] The color transitions of phenolphthalein and *o*-cresolphthalein occur in approximately the same zone of hydrogen ion concentration. The latter indicator may be used more advantageously in deeply colored media since its color changes are more brilliant than those of phenolphthalein.

and Eckstein (14) gives as satisfactory results as any in solutions where the tendency to foam is abnormally great.

A satisfactory procedure when working with bacterial cultures is to grow them in Florence Pyrex flasks of 300 cc. capacity, employing 100 cc. of medium per flask. Connect the flask with an upright water-cooled condenser so that the discharge of the condensate is rapid and complete. A rubber stopper covered with tin-foil provides a clean and vapor-tight connection between flask and condenser.

Adjustment of Reaction.—The culture or medium containing indole is adjusted by the addition of normal NaOH to the desired degree of hydrogen ion concentration as shown by phenolphthalein and checked by thymolphthalein. This may be accomplished, *where extreme accuracy is desired*, by pipetting 10 cc. of the medium into a test-tube, adding five drops of the 0.02 per cent indicator solution, and then adding alkali drop by drop until the depth of color matches the depth of color produced by the same quantity of the indicator in 10 cc. of a standard buffer mixture. This standard buffer mixture may be prepared to represent a true hydrogen ion concentration of pH 9.2, or any other decided upon for the distillation, by following the method proposed by Clark and Lubs (15). If the 10 cc. of medium represent one-tenth of the total volume to be distilled, then by multiplying the number of drops or cc. of alkali required for the one-tenth portion by nine, the quantity of alkali necessary for the remaining 90 cc. to reach the same pH will be determined. A burette is convenient for holding and measuring the alkali. The 10 cc. portion containing the indicator and alkali may be returned to the flask for distillation.

If no standard buffer mixture is at hand it is possible to adjust the reaction directly in the flasks, and *this is to be recommended in all cases of laboratory routine and when working with pathogenic organisms.* All that is necessary in this case is to run normal alkali into the solution until phenolphthalein shows a full red and thymolphthalein shows no blue. One drop of indicator as it falls on the surface of the medium is sufficient to indicate these pH values when observed at the point of contact. The writer has successfully used a porcelain-spot test-plate for determining the reaction. Two or more drops of media and one drop of the 0.02 per cent indicator solution give excellent definition.

The distillation is continued until all but about 10 cc. has been driven over, the distillate being collected in a 100 cc. volumetric flask and made up to the mark. This represents the total indole from the culture.

Determination of Indole.

Preparation of Standards.—10 mg. of pure indole are dissolved in 100 cc. of distilled water, warming the water slightly if necessary. A set of 10 test-tubes $\frac{1}{2}$ inch in diameter and of uniform thickness and color are arranged and numbered to receive portions of the above indole standard delivered from a burette graduated in 0.1 cc. (0.01 cc. if obtainable). It has been found useful to arrange the following concentrations in the tubes: 0.02, 0.04, 0.06, 0.08, 0.10, 0.12, 0.14, 0.16, 0.18, and 0.20 mg. of indole respectively. Water is now added to the tubes to approximate a total of 10 cc. To each tube are added two drops of a 1.0 per cent solution of sodium nitrite ($NaNO_2$) and five drops of concentrated sulfuric acid. Each tube is carefully shaken by inversion. After standing for about 5 minutes each tube is extracted with three portions (3 cc. per portion) of isoamyl or isobutyl alcohol. These three extractions are combined in one test-tube and made up to exactly 10 cc. with the pure extractant; this represents the total nitroso-indole from the respective indole standards. Both isoamyl and isobutyl alcohols, under the conditions of the test, form clear strata above the water portion without emulsification and can be drawn off by means of a pipette, the stem of which is bent at right angle and possesses a soft rubber tube. The test-tubes can be marked at the 10 cc. volume point.

These alcoholic nitroso-indole standards are thoroughly reliable for a working period of a week or longer. They may be placed in subdued light when not in use. They have been observed for a period of 2 months in strong light and the only change observed was a slightly increased detail of the absorption bands. No new bands were noticed; neither was there shifting of the old. No change was apparent to the eye.

Details of Test.—An aliquot of the distillate from the indole separation is pipetted into a test-tube of convenient size; the aliquot may be 10, 15, or 20 cc., or larger, just so that no more

than 0.20 mg. of indole is present in it. If the quantity of indole is known to be small then a larger aliquot is necessary, since there must be at least 0.01 mg. in order to compare with the above mentioned standards. Of course any similar set of indole standards may be made up, employing different concentrations. For instance Nonnotte and Demanche advised the use of the drop system in measuring the indole solutions for the nitroso-indole standards. This latter system is obviously very faulty, and was found to yield varying results.

The aliquot of distillate is measured into a test-tube and two drops of 1.0 per cent $NaNO_2$ solution are then added, this being followed by five drops of concentrated sulfuric acid. The test-tube is shaken and allowed to stand about 5 minutes for the nitroso reaction to approach equilibrium. It is then extracted with three portions (3 cc. per portion) of isobutyl or isoamyl alcohol as before, and each portion is drawn off with a pipette and run into a test-tube of the same dimensions and quality as those which hold the standards. This tube should previously be marked at the 10.0 cc. volume point, so that after adding the last portion of extraction it may be made up to the mark and compared with the standards. It is advisable to run duplicates on the distillates at the same time. With a little experience it is possible to interpolate very accurately between successive 0.02 mg. standards by matching the relative intensity.

If it is desired to work with smaller portions of cultures or biological products the same procedure as outlined above may be followed, with the added precaution of diluting the mixture or culture to 100 cc. before distilling. In this case it may be necessary to work with the entire distillate, when a small separatory funnel will be found convenient.

Comparison of the Nitroso-Indole Method with the Beta-Naphtha-Quinone-Sodium-Monosulfonate Method as Applied by Bergeim.

2.5 mg. of Eimer and Amend indole were dissolved in 200 cc. of distilled water. This was used as standard for the comparative determinations, portions of which were carefully measured into tubes from which the contained indole was extracted. Chloro-

form, as suggested by Bergeim, was used as an extractant when following his method. Isobutyl (primary) alcohol was used to extract the nitroso-indole. A 1.0 cc. solution of indole containing 0.10 mg. was diluted, treated with beta-naphtha-quinone-sodium-monosulfonate[2] and extracted with chloroform, and the total chloroformic extract was made up to 15 cc. This was used as

TABLE I.

Description of experiment.	Indole.			
	Bergeim method.		Zoller method.	
	In portion.	Found.	In portion.	Found.
	mg.	*mg.*	*mg.*	*mg.*
15 cc. portions of prepared indole solution	0.1375	0.1362 0.1365	0.1375	0.1350 0.1350
10 cc. portions of prepared indole solution	0.1250	0.1246 0.1248	0.1250	0.1300 0.1250
200 cc. culture of *B. coli* grown on medium for 30 hrs.; 100 cc. by Bergeim method, 100 cc. by Zoller method*		0.465 0.468		0.478 0.476
10 cc. portion of preparation + 1.0 per cent of phenol			0.1250	0.1275 0.1250
10 cc. portion + 1.0 cc. of 0.1 N NH_3.			0.1250	0.1300 0.1250

* Time required for Bergeim's separation and test, 3.5 hrs.; for Zoller's separation and test, 1.0 hr.

standard in a Duboscq colorimeter for all determinations which were based upon Bergeim's method.

As a further test of the practicability of the proposed method for estimating indole, a culture of *Bacillus coli* (Lab. No. fg) was grown in 500 cc. of a medium of 1.0 per cent Difco peptone, 0.5

[2] The writer uses this opportunity to thank Dr. O. Bergeim, of the Jefferson Medical College, Philadelphia, Pa., for a liberal quantity of beta-naptha-quinone-sodium-monosulfonate furnished for indole determinations.

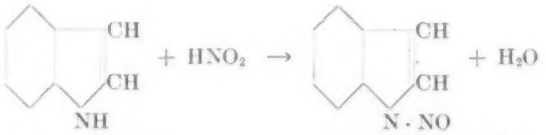
per cent K_2HPO_4, and 0.1 per cent dextrose for 30 hours. Two portions of 100 cc. each were simultaneously removed from this culture, and the indole was removed from one portion by Bergeim's double distillation method, and from the other portion by the scheme involving adjustment of reaction and distillation as outlined in this paper. The indole was determined in portions of the separate distillates and calculated to milligrams of total indole in 100 cc. of the culture. The results are presented in Table I.

DISCUSSION.

It should be remembered that skatole is not an infringing danger to the success of the proposed method. Fischer (16) and Brieger (17) as well as Salkowski pointed out that nitroso-skatole, if formed, was white and insoluble in the acid solution in which it is formed. The writer has found it to be only slightly soluble in isobutyl alcohol, and it did not give absorption in the visible portion of the spectrum. It seems likely therefore that skatole would not interfere in the determination.

Indoleacetic acid, as previously mentioned, reacts similarly with nitrous acid to give a red nitroso-indoleacetic acid. This may be responsible for the "cholera-red" reaction of Nencki (18) as well as the urorosein. Since indoleacetic acid is non-volatile with water vapor it is eliminated as a danger to the accuracy of the test.

It is very likely that the formation of nitroso-indole follows the reaction



Indole. Red nitroso-indole.

Nitroso-indole is readily soluble in methyl alcohol and can be crystallized from it in flesh-colored, shiny leaves or flakes. These seem to remain unchanged for long periods of time and suggest the possibility of preparing standards directly from the purified crystals. The crystals melt at 170°C. (uncorrected), with decomposition.

The nitroso-indole reaction, when supplemented with the alcohol extraction, becomes an exceedingly sensitive and convenient way of detecting the presence of indole. The relative sensitivity of Herter's naphthaquinone reaction and the nitroso reaction were found to be respectively 1:2,000,000 and 1:1,500,000. The extraction of the excess of the reagent in the former case by the chloroform reduces noticeably its sensitiveness. It is also necessary that the naphthaquinone reagent be freshly prepared in order to secure best results. With the nitroso-indole test the only precautions necessary are the proper concentrations of nitrite solution and acid. Excess nitrite or acid tends to displace the sensitiveness. The most effective working limits for the reaction as applied seem to be those given in the above method. It is important to know that the alcoholic solutions of nitroso-indole are much more stable than the aqueous solutions.

Recently a number of investigators have used the vanillin test proposed by Steensma (19). Blumenthal (20) reported in 1909 a very careful survey of the color reaction between various aromatic aldehydes, including vanillin, and indole and skatole. While many of these aldehydes yield beautiful color reactions with indole and skatole, their sensitiveness is greatly impaired because of the high reactivity of the aldehydes for other compounds such as phenols and ammonia. The danger of performing the vanillin test in cultures of organisms is at once apparent. The yellow-orange color which develops when indole and vanillin are brought together in acid solution is very readily masked, as well as duplicated by other substances. When the test is applied to the indole distillate from such cultures its delicacy is only slightly more enhanced and even then is not so discriminating between the homologues as the nitroso-indole, *p*-dimethyl-amino-benzaldehyde, or naphtha-quinone-monosulfonate reactions. The arguments brought forward for its extreme delicacy are mainly fallacious in view of these facts. The writer, therefore, believes that a note of warning should be sounded against its promiscuous use in bacteriological work.

The rate at which the equilibrium of the nitroso-indole reaction is reached depends upon the factors of concentration and temperature. It is notable that with temperatures above normal, 30 to 90°C., the full depth of color is obtained in a few seconds. Higher

temperatures tend to destroy the colored chromogen when in aqueous solutions. In pure isobutyl alcohol the color is not destroyed even after several hours' heating at temperatures around 100°C. Inasmuch as indole is volatile with water vapor, the rate being chiefly dependent upon temperatures, it is unwise to conduct the test or determination at temperatures above 30°C.

It seems that the features of readily available reagents and simplicity and speed of performance are in themselves sufficient to recommend the proposed method to any one. In addition to these points it is well to consider the fact that indole occurs in biological media in the extremes of concentrations and that the foregoing method as devised is particularly adapted to measure these extremes. Since the evidence reported shows it to check favorably with the best from the standpoint of accuracy and its sensitiveness to be less affected by contaminating substances, it should find very general application.

SUMMARY.

1. The limitations of the nitroso-indole reaction of Baeyer have been studied with the idea of improving and standardizing the Nonnotte and Demanche application of it to the quantitative determination of indole.

2. The method evolved is simple, reliable, and rapid, and requires only the reagents and apparatus common to most laboratories.

3. The indole is separated from the parent mass by one direct distillation. The limitations of this distillation have been reported elsewhere (13) and are dependent upon the hydrogen ion concentration of the medium.

4. Use of the nitroso reaction, when accompanied by an alcohol solvent, for testing the distillate from biological media for the presence of indole is advocated. A warning is sounded against the use of the vanillin-acid test for indole.

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INFLUENCE OF HYDROGEN ION CONCENTRATION UPON THE VOLATILITY OF INDOLE FROM AQUEOUS SOLUTION.*

By HARPER F. ZOLLER.

(From the Research Laboratories of the Dairy Division, Bureau of Animal Industry, United States Department of Agriculture, Washington.)

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The methods in common practice for the separation of indole from biological mixtures stipulate the addition of either acid or alkali of strong concentration to the flasks of suspended material from which the indole is to be driven by steam distillation or direct boiling. The purpose of such treatment is, doubtless, to prevent the volatilization of interfering substances by neutralizing them *in situ*. Not infrequently one finds the direction to double distill; *i.e.*, distill first from alkali with steam and then acidify the distillate and redistill with steam or *vice versa*. In the procedures published in the literature no apparent thought is given to the final reaction of the medium, or to the reserve alkalinity or acidity which it possesses. No attempt is made to identify the concentration of the acid or alkali with its chance effect upon the indole molecule, either internally or externally. It is the purpose of this paper, therefore, to report the results of a study aimed toward the defining of standard conditions for the separation of indole from various media. In projecting the study two objectives were sought. One was to determine whether steam distillation could be safely abandoned for direct distillation. The other was to ascertain whether or not the conditions for such a separation would destroy the indole molecule.

Since indole is an aromatic imine possessing basic properties—although the degree of basicity is greatly modified by the orien-

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tation within the double-ring structure (as proved by the catalytic reduction of indole into *o*-toluidine and methane)—(1) we should expect it to form associated compounds with strong acids. In this expectation we are not disappointed, for the compounds indole nitrate (2) and indole hydrochloride (3) have been isolated. The methods of isolation and their properties suggest that these compounds are very loosely associated. The fact that indole may be removed from a strong acid solution by steam, when proper cautions are observed, tends to justify this assumption.

As previously mentioned, the concentration of acid employed in the isolation of indole has been rather high, although varying through a wide range. It is well known that a hydrogen ion concentration maintained at 1.0×10^{-6} is amply sufficient to prevent the volatilization of ammonia from aqueous solutions. In many instances in the practice of distilling indole with water vapor the hydrogen ion concentration is in excess of 1.0×10^{-1}. Now it was thought that the rate at which indole volatilized from aqueous solutions, adjusted to definite initial hydrogen ion concentration, might be of value in defining the conditions under which the distillation of indole could be safely conducted.

One could anticipate, inasmuch as indole possesses feebly basic properties, that it would volatilize more freely from an alkaline solution. To what extent the alkalinity might be carried without producing disintegration of the indole molecule has never been, to the writer's knowledge, published. This might be roughly ascertained by determining the indole distilled and that remaining in the distillation flasks after a series of diverging alkalinities had been run. This, of course, would necessitate the assumption that the products resulting from the action of alkali on indole do not act like indole toward the test reagents. Such an assumption is only partially justifiable. In very strong alkali solutions indole converts first to indoxyl and thence to indigo blue, following the course of a true oxidation. Neither of these substances would confuse the nitroso test for indole.

With these considerations in mind and with a rather rapid method of determining indole at hand (4), the following study was made.

Experimental A.

Twelve Florence-shaped, 250 cc., Pyrex flasks were provided. Into each were poured 90 cc. of distilled water containing 1.0 mg. of indole. The hydrogen ion concentration of each solution was initially adjusted to the respective pH values, the term $pH = \log \frac{1}{\text{hydrogen ion}}$ being that adopted by Sörensen (5).

Flask No.	pH	Indicator.	Flask No.	pH	Indicator.
A	1.0	Thymol blue.	G	6.0	Bromocresol purple.
B	2.0	" "	H	7.0	Phenol red.
C	3.0	Bromophenol blue.	I	7.6	" "
D	4.0	" "	J	8.0	Cresol "
E	4.4	Methyl red.	K	9.0	Cresolphthalein.
F	5.0	" "	L	10.0	Thymolphthalein.
			M	10.5	"

In adjusting the reaction of the solutions use was made of a 0.1 N solution of HCl and 0.1 N disodium hydrogen phosphate ($Na_2HPO_4.2H_2O$) for the range of pH 1.0 to 8.0, while for the range of pH 9.0 to 10.5 a 0.1 N NaOH solution in conjunction with the Na_2HPO_4 solution. Burettes carried the solutions mentioned. The indicators developed and reported by Clark and Lubs (6) were employed for accurately determining the initial pH points. In addition, thymolphthalein served to determine the concentrations pH 10.0 and 10.5. The total volume of solution within the flasks was finally adjusted to 100 cc. with distilled water. They were then placed upon an iron wire gauze over a free flame of a Bunsen burner and the contents directly distilled through glass connections into an upright, short tube (water jacket 7.0 inches) condenser. The condensate was collected in 50, 25, and 15 cc. portions, and in the noted order, graduated cylinders acting as collectors. The quantity of indole in each portion was determined by the nitroso-indole method (4). The amount of indole remaining in the flasks was also determined in the same manner.

It should be mentioned that a glass tube ebullator was placed in each flask before distilling in order to insure a constant flow of vapor into the condenser. The more alkaline solutions, pH 9.0 to 10.5, were carefully watched to prevent foaming. It was undesirable in this study to add chemical foam reducers since the majority of them (amyl, octyl, and heptyl alcohol and phenyl ether) are solvents for indole and immiscible with water; hence their use would introduce the question of effect upon the volatility of indole.

The results of the experiment above are included in Table I. The values in the last column, expressing the per cent of volatility of indole from aqueous solution at different pH values are shown graphically in Fig. 1.

TABLE I.

Reaction of solution.	Indole in aqueous solution.					
	Flask.	50 cc.	25 cc.	15 cc.	Flask.	Distilling in first 75 cc.
pH	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*per cent*
1.0	1.000	0.610	0.205	0.13	0.050	81.5
2.0	1.000	0.590	0.200	0.140	0.065	79.0
3.0	1.000	0.605	0.220	0.125	0.050	82.5
4.0	1.000	0.570	0.205	0.140	0.090	77.5
4.4	1.000	0.585	0.190	0.125	0.100	77.5
5.0	1.000	0.585	0.210	0.105	0.095	79.5
6.0	1.000	0.690	0.205	0.080	0.030	89.5
7.0	1.000	0.785	0.185	0.025	0.000	97.0
7.6	1.000	0.855	0.130	0.015	0.000	98.5
8.0	1.000	0.860	0.135	0.010	0.000	99.5
9.0	1.000	0.906	0.090	0.000	0.000	99.6
10.0	1.000	0.915	0.086	0.000	0.000	100.1
10.5	1.000	0.912	0.084	0.000	0.000	99.6

Since it might be construed that the above values would not be approached in complex media, although such media possessed corresponding pH values, it was, therefore, desirable to repeat the above experiments, employing a protein suspension in lieu of the aqueous solutions of indole. Consequently a medium was prepared which possessed approximately the following compositions: 2.0 per cent Difco peptone, 0.1 per cent dextrose, and 1.0 per cent K_2HPO_4.

50 cc. of this medium were measured into each of the above flasks. 1.0 mg. of indole was added in the form of 10 cc. of a 0.1 per cent solution. The corresponding pH values were adjusted in the various flasks, eliminating pH 4.4 and 7.6. The final volume of solution was made up to 100 cc. and distilled as in the previous study. Flasks holding solutions at pH 9.0, 10.0, and 10.5 received 1.0 cc. of phenylether each to prevent excessive foaming. The results are reported in Table II.

TABLE II.

Reaction of suspension.	Indole in protein suspension.			
	Flasks.	50 cc.	25 cc.	Distilling in first 75 cc.
pH	*mg.*	*mg.*	*mg.*	*per cent*
1.0	1.000	0.585	0.205	79.0
2.0	1.000	0.600	0.200	80.0
3.0	1.000	0.595	0.210	80.5
4.0	1.000	0.595	0.205	80.0
5.0	1.000	0.590	0.220	81.0
6.0	1.000	0.620	0.240	86.0
7.0	1.000	0.725	0.200	92.5
8.0	1.000	0.875	0.115	99.0
9.0	1.000	0.890	0.105	99.5
10.0	1.000	0.910	0.080	99.0
10.5	1.000	0.905	0.095	100.0

No attempt was made herein to determine the distribution of indole beyond the first 50 and 25 cc. portion of distillate. It was assumed that no greater destruction of indole would occur in the mixed medium than in the purer solutions. Considerable ammonia was evolved from the last two flasks. The rate of volatilization of the indole was plotted against the pH and is reproduced in Fig. 2.

Experimental B.

In order to further establish the rate of volatility another set of aqueous solutions of indole was prepared according to methods followed under A, except that 2.0 mg. of indole were dissolved in a total volume of 200 cc. The range of pH was, with the exception of 4.4 and 7.6, the same as in Table I. Each solution of indole was placed in a gas-washing bottle possessing gas-tight junctures. This bottle was in turn set into a water bath (the water bath was maintained at 50°C. throughout the experiment) and then connected to a train of three other bottles B, C, and D. Bottle B contained distilled water, C 50 per cent NaOH, and D an improvised glass gas meter so constructed that 20 cc. of air were passed at each dump. Communication was now established between the bottle carrying the indole solution and the vacuum line, and the rate of suction so adjusted that 1,000 cc. of CO_2-free air passed through the indole solution each minute for 60 minutes.

After aerating each solution for 1 hour they were again adjusted to 200 cc. and the indole content was determined in an aliquot. The per cent of loss of indole is plotted against the pH and is represented in Fig. 3.

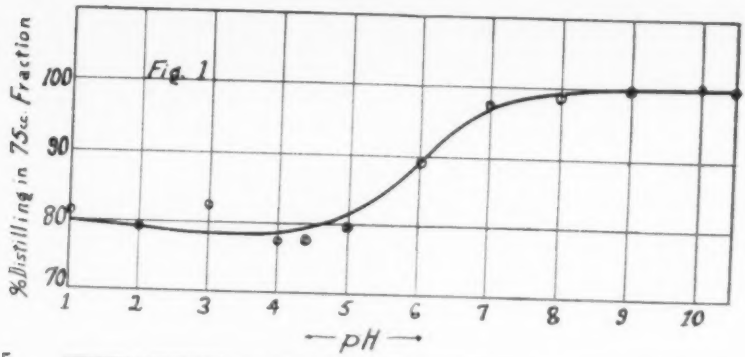
Fig. 1
% Distilling in 75cc Fraction
100
90
80
70
1 2 3 4 5 6 7 8 9 10
← pH →

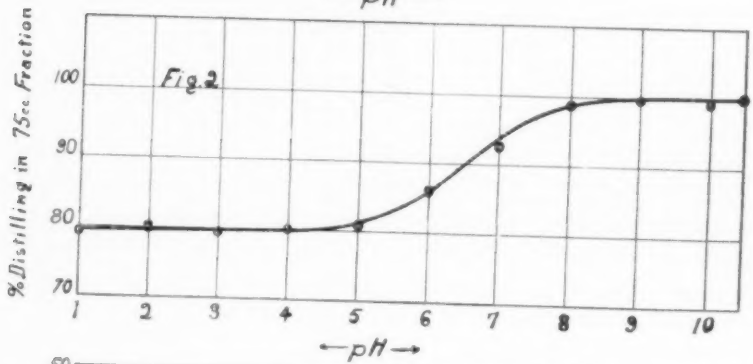
Fig. 2
% Distilling in 75cc Fraction
100
90
80
70
1 2 3 4 5 6 7 8 9 10
← pH →

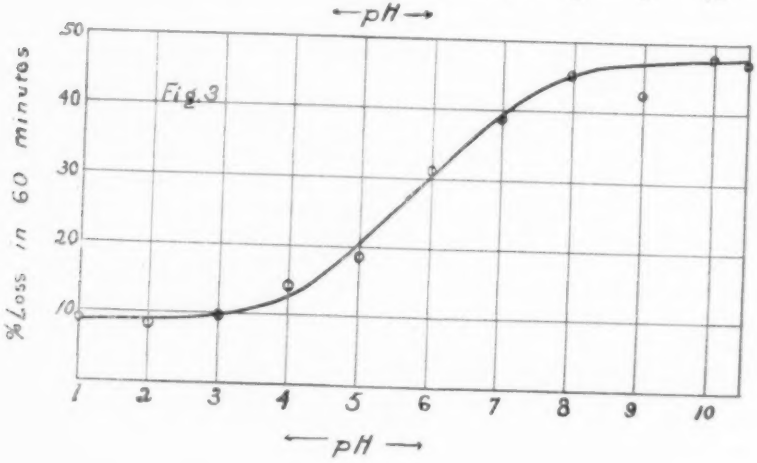
Fig. 3
% Loss in 60 minutes
50
40
30
20
10
1 2 3 4 5 6 7 8 9 10
← pH →

DISCUSSION.

It is clear from the published results that the tendency is towards an increased volatility of indole from solutions possessing a hydrogen ion concentration of 1.0×10^{-8} and less; or in terms of Sörensen's nomenclature, pH 8.0 to 10.5. There was no reason for the studying of acidities or alkalinities of a higher order, since the ranges of those investigated are extreme enough for all practical purposes.

The comparative regularity of the distillations as performed, and the completeness of the volatilization of the indole in the first 75 cc. of distillate from aqueous solutions possessing an initial pH of 8.0 and higher, suggest that the practice of steam distillation is unnecessary. A mixture containing indole and adjusted to a pH of 9.0 to 10.0 will not volatilize any phenols, but will permit some ammonia, skatole, and other volatile bases to be driven over with water vapor. Ammonia may be removed by shaking out with exchange silicate (permutit) or yellow mercuric oxide (7), while permutit will also remove small amounts of certain alkylamines. Neither of these absorbents will remove indole or skatole. A further test pointing to the regularity of the distillation was conducted by distilling 100 cc. of a solution containing 1.0 mg. of indole, buffered at an initial pH of 9.0, from a Duclaux still patterned after that designed by Gillespie and Walters (8). Nine successive 10 cc. portions of the distillate were collected and separately analyzed for indole. The ninth fraction was free from indole while the eighth contained less than 0.01 mg.

The application of this principle of direct distillation of indole from crude mixtures has been practiced in connection with some bacteriological work in this laboratory and with satisfying results. The method as applied is discussed in another paper (4). It may be remarked that the method is much preferable to the cumbersome one of steam distillation, especially when dealing with pathogenic organisms.

SUMMARY.

1. The range of most rapid volatilization of indole from the aqueous solutions studied is from pH 8.0 to 10.5.

2. Hydrogen ion concentrations greater than 1.0×10^{-6} caused a decrease in volatility of indole with water vapor, probably due to the formation of weakly associated combinations between indole and acid in aqueous solution.

3. Hydrogen ion concentrations of the order of pH 10.5 have no appreciable destructive action on the indole molecule under the conditions of the study.

4. The results from the experiments performed suggest that the practice of steam distillation can be supplanted by direct distillation with equal accuracy when the reaction of the solution is taken into account. This direct method of distillation has been practiced in a routine investigation in this laboratory and found reliable and commendable.

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AVAILABILITY OF CARBOHYDRATE IN CERTAIN VEGETABLES.

By W. H. OLMSTED.

(*From the Laboratories of the Departments of Medicine and of Biological Chemistry, Washington University School of Medicine, St. Louis.*)

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The extensive use of certain vegetables of low carbohydrate content, such as spinach, cabbage, cauliflower, and lettuce, in the diet of patients suffering from diabetes during the determination of their carbohydrate tolerance makes it desirable to know with considerable accuracy the amount of sugar-forming substance which these foods contain. The recorded analyses permit only a doubtful calculation of the desired information, because among other reasons much of the carbohydrate is "fiber" which presumably is not digested or absorbed by man; the availability of the starch may be more or less uncertain depending upon the extent to which it is liberated from its protecting cell structure by cooking, mastication, and digestion; and because certain organic acids, which by hydrolysis and reduction methods are not determined as carbohydrate, may form sugar after absorption from the intestine. Furthermore, published analyses[1] usually give carbohydrate as determined only by difference, and the combined errors thus fall on this constituent. While such figures represent more or less correctly total carbohydrate, with or without fiber, there is no assurance that they indicate the amount of carbohydrate available to the body when the materials are eaten. Thus, Lusk[2] fed cauliflower to a phloridzinized dog without detecting sugar production, yet by analysis cauliflower contains 3.7 per cent carbohydrate.

[1] Atwater, W. O., and Bryant, A. P., *U. S. Dept. Agric., Bull. 28*, revised, 1906.

[2] Lusk, G., *Am. J. Physiol.*, 1910–11, xxvii, 467.

It was, therefore, decided to determine by more direct methods the sugar-forming material in those vegetables which are commonly used in the dietaries of diabetics in order to be able to calculate more exactly the available carbohydrate in such diets.

Four methods have beeen used for the determination of carbohydrate in foodstuffs. First is the method most commonly used by which carbohydrate is calculated by difference after direct determination of fat, protein (N × 6.25), ash, water, and insoluble fiber; second, direct hydrolysis by mineral acid and the subsequent determination of reducing sugar; third, preliminary hydrolysis of starch by diastase to dextrins, maltose, and glucose, followed by copper reduction and determination by polariscope or by acid hydrolysis for the conversion to glucose which is then determined by reduction; and fourth, analysis by means of the phloridzinized animal. We have used the last two methods named.

The use of the phloridzinized dogs should give results indicating the total available sugar-forming substance, including sugar formed by such substances as protein and organic acids; while the results of the diastase method should indicate only reducing sugars, preformed, and from the hydrolysis of starch.

Analysis in Vitro by Diastase and Copper Reduction.

The direct hydrolysis of the vegetable material by boiling hydrochloric acid is objectionable because of the hydrolysis of the indigestible fiber and because as shown by Davis and Daish[3] 5 or 6 per cent of the maltose may be decomposed and lost.

The milder and more selective action of a diastase as the hydrolytic agent for the starch seems therefore preferable, and has been used for our analyses. Of the commercial diastases available, that of the *Aspergillus oryzæ*, the "taka-diastase," proved the most satisfactory, in that hydrolysis was more complete. Results with a 10 per cent extract of malt as recommended in the official methods[4] gave only 90 to 95 per cent with pure starch. Davis and Daish[3] used taka-diastase successfully in their determinations of starch in certain leaves.

[3] Davis, W. A., and Daish, A. J., *J. Agric. Sc.*, 1914, vi, 152.

[4] *J. Assn. Offic. Agric. Chem.*, 1916, ii, pt. 1, 110.

For the determination of the sugar formed by the action of diastase, Davis and Daish used the combined methods of reduction and optical activity of the solutions. We have preferred to employ a secondary hydrolysis of the maltose and any residual polysaccharide by heating with a minimum concentration of acid and to determine the resulting glucose by copper reduction. While this acid hydrolysis probably leads to some decomposition of sugar, the loss under the conditions used is not greater than about 2 per cent and is probably within the limit of error of other stages in the procedure. The acid hydrolysis was carried out on the individual portions taken for analysis in centrifuge tubes placed in boiling water bath under which condition there is less destruction of sugar than occurs in boiling with a reflux condenser.

The principles in the determination were: The thorough trituration and maceration of vegetable samples; the action of 0.1 gm. of taka-diastase on watery samples at 37°C. for 18 hours; filtration, hydrolysis, and the determination of glucose by Fehling's solution, using Bertrand's method for titration of cuprous oxide.

Procedure.

Samples of the vegetables were carefully selected, put through the finest food chopper, and 20 to 50 gm. weighed out, care being taken to retain the water squeezed out in grinding. These samples were put into a flask with reflux condenser and boiled on a hot plate 1 or 2 hours in 100 to 150 cc. of water. After cooking the sediment was macerated to a paste in a mortar. The only vegetable which offered any difficulty was lettuce and it was found impossible to macerate the leaves, even after prolonged boiling. After combining mother liquor and paste, each sample was washed into a 250 cc. flask and boiled again in a water bath 15 minutes, then cooled to 37° C., and 0.1 gm. of taka-diastase was added to each flask. All samples were incubated 17 hours at 37° C. with a few drops of toluene as a preservative. After incubation the flasks were made up to volume, well shaken, and 100 cc. taken. 5 cc. of a molecular solution of neutral lead acetate were added to precipitate tannins and gums. Samples were filtered, the filtrate being tested with lead for completeness of precipitation. 10 cc. of filtrate—equaling 9.52 cc. of the original—were taken for determination.

The hydrolysis of sugars, reduction of copper, and the titration by Bertrand's method were done in 50 cc. centrifuge tubes.[5] To 10 cc. of the

[5] Schaffer, P. A., *J. Biol. Chem.*, 1914, xix, 285.

TABLE I.

Analysis of Starch by Taka-Diastase and Hydrolysis.

Two samples of Kahlbaum's arrowroot starch, dry weight 0.88 gm., were washed into 250 cc. volumetric flasks with 150 cc. of water. Gelatinized 15 minutes in boiling water bath; cooled to 37°C. and 0.1 gm. of commercial taka-diastase added. Samples incubated at 37° for 20 hours.

100 cc. of starch-diastase mixture were taken to which were added 5 cc. of a molecular solution of neutral lead acetate.

10 cc. of filtrate, representing 9.52 cc. of the original, were taken for determination (36.85 mg. of dextrose).

Samples hydrolyzed 2½ hrs., concentration of acid 0.7 N.

0.04 N permanganate.	Copper.	Copper corrected for blanks.	Glucose.	Theoretical.
cc.	*mg.*	*mg.*	*mg.*	*per cent*
31.3	79.3	73.2	36.9	100.1
31.2	79.0	72.9	36.8	99.8
31.0	78.5	72.4	36.5	99.0
31.4	79.4	73.3	37.0	100.4

TABLE II.

Summary of Analyses by Taka-Diastase.

Vegetable.	Weight of the sample.	No. of samples.	Average glucose in aliquot (9.52 cc.) samples.	Average glucose in samples.	Carbohydrate as glucose.
	gm.		*mg.*	*gm.*	*per cent*
Potato	5	6	35.5	0.930	18.6
Lettuce	50	4	18.9	0.495	1.0
Cabbage	20	4	33.5	0.877	4.38
Cauliflower	30	6	32.1	0.840	2.80
Cabbage, thrice boiled	30	5	4.7	0.123	0.4
Cauliflower, " "	30	6	10.2	0.267	0.8

TABLE III.

Analyses of Vegetables by Taka-Diastase and by Atwater and Bryant Compared.

	Taka-diastase.	Atwater and Bryant.
Cabbage	4.4	4.5
" thrice cooked	0.4	
Cauliflower	2.8	3.7
" thrice cooked	0.8	
Lettuce	1.0	2.2

filtrate were added 2 cc. of 7 N hydrochloric acid and water to give a volume of 20 cc. The final concentration of acid was thus 0.7 N. The tubes were placed in a gently boiling water bath 2½ hours. After hydrolysis the acid was almost neutralized with a measured amount of strong sodium hydroxide, 20 cc. of mixed Fehling's solution were added, and the tubes heated in a boiling water bath exactly 10 minutes. Tubes were centrifuged 3 minutes, the excess Fehling's solution was decanted, and cuprous oxide washed twice with 20 cc. of water, centrifuging and decanting each time. After decanting following the last washing, copper was dissolved in 1 to 2 cc. of strong ferric sulfate-sulfuric acid and titrated with 0.04 N potassium permanganate solution. Blanks were always run on 0.1 gm. of taka-diastase and on the mixed Fehling's solution, the titration of blanks being deducted from titration of cuprous oxide of the sample. The number of cc. of 0.04 N permanganate used multiplied by 2.54 gives the mg. of glucose present in 9.52 cc. of sample. The Bertrand titration was checked by titrating solutions of pure anhydrous glucose.

The use of centrifuge tubes simplifies the process considerably but limits the amount of mixed Fehling's solution that can be used. Experiments showed that 20 cc. of mixed Fehling's solution gives accurate results when between 30 and 40 mg. of dextrose are present. So in the determinations the amount of the sample was so arranged as to give amounts of dextrose within these limits as near as possible.

This method applied to pure, dry, arrowroot starch gave results shown in Table I. As applied to potato, cabbage, cauliflower, and lettuce the method gave results shown in Table II.

The results of analyses of cabbage, cauliflower, and lettuce by the use of taka-diastase were lower than the accepted analyses of Atwater and Bryant; this was especially true of lettuce. Table III is a comparison of analyses.

The extensive use of thrice cooked cabbage and cauliflower in the treatment of diabetes suggested the analyses of these as prepared in the diet kitchen according to Joslin.[6] The results show that 60 to 90 per cent of the carbohydrate is lost in the cooking water.

Experiments with Phloridzinized Animals.

The phloridzinization of dogs was conducted after the method of Sansum and Woodyatt;[7] namely, 48 hours of complete fasting followed by 1 gm. of phloridzin in oil subcutaneously every 12

[6] Joslin, E. P., Treatment of diabetes mellitus, Philadelphia and New York, 1916.

[7] Sansum, W. D., and Woodyatt, R. T., *J. Biol. Chem.*, 1916, xxiv, 327.

hours and adrenalin, 0.04 mg. per kilo of body weight of animal, subcutaneously every 6 hours.

Sansum and Woodyatt,[8] Lusk, Janney,[9] and others have previously used phloridzinized animals as a method of carbohydrate analysis. The animals were kept in clean metabolism cages and catheterized at the end of each experimental period. The urines were preserved with toluene and made up to 1,000 or 1,500 cc.

Nitrogen in urine was determined by Kjeldahl and sugar by the same technique used with plant extracts, clearing with lead acetate, reduction of Fehling's solution, and titration of copper by Bertrand. All urines were read in a polariscope but more accurate D: N ratios were obtained by determining glucose by reduction.

The urine from some dogs gave low glucose figures when calculated from polariscopic readings. In other animals the difference between the results calculated from polariscope and reduction was not great. In Tables IV and V glucose calculated by both methods is given. In Table IV the difference is not very great, but in Table V it is considerable. It will be seen that the D: N ratios calculated from polariscopic values are low, being below 3.00. The presence of levorotatory substances in dog urine is well known and for that reason the determination of glucose by reduction is more accurate. Determinations by polariscope should not be accepted in such work.

Feeding 200 to 500 gm. of bulky vegetables presented some difficulty. The animals refused to eat them of their own volition, but by mixing melted lard with the cooked vegetables and placing the mixture in the animal's mouth, it would always be quickly and often eagerly swallowed. Any liquor left after feeding the bulky material was fed by stomach tube. No vomiting of feedings was experienced. The vegetables were prepared in the same manner used in analysis by diastase, care being taken to boil down water to a minimum. The results are, therefore, for cooked vegetables. If eaten raw the amount of starch digested would doubtless be less.

[8] Sansum, W. D., and Woodyatt, R. T., *J. Biol. Chem.*, 1916, xxiv, 23.
[9] Janney, N. W., *J. Biol. Chem.*, 1915, xx, 321.

The extra sugar in all experiments was calculated by the usual method from the average of the starvation D:N ratios of the periods before and after the feeding periods. At the foot of each table are given these ratios. The starvation ratios multiplied by the nitrogen elimination of the feeding periods gives the sugar arising from the protein and this amount subtracted from the sugar excreted during feeding period gives the extra glucose arising from carbohydrate material fed.

The periods where vegetables were fed were made 24 hours in length. It was found that 24 to 30 hours are required for the elimination of all the extra glucose. This slow elimination of extra sugar from such sources of carbohydrate must be taken into account in any experiments where vegetables are fed to phloridzinized animals. In some of the experiments the amount of extra glucose was very small. But the large amount of material, and careful verification of analysis and calculations have convinced us that these amounts of glucose arose from the material fed.

Table IV gives the result from a dog to which was given glucose as a control. The calculation gives a recovery of from 94 to 107 per cent of the sugar fed. It is of interest to note that the sugar fed in Period IV had been racemized by boiling alkaline phosphate solution until the solution was no longer optically active. In the organism, it was, however, converted again quantitatively into dextroglucose. The results indicate satisfactory recovery of available carbohydrate.

Sansum and Woodyatt[8] obtained from 50 to 75 per cent of dextrose fed by mouth to phloridzinized dogs; Csonka[10] recovered 100 per cent in his experiments.

Table V shows recovery of 17.3 dextrose from 500 gm. of cauliflower or 3.4 per cent of weight. Although Lusk[2] found no extra sugar after 20 gm. of cauliflower fed to a phloridzinized animal, the small amount of ingested material may well account for his result. By analysis 2.8 per cent of weight of cauliflower was found to be carbohydrate.

[10] Csonka, F. A., *J. Biol. Chem.*, 1916, xxvi, 93.

TABLE IV.

Dog 7 Phloridzinized after 2 Days of Fasting; Weight, 13 Kilos.

10 gm. of sugar given in Period IV were boiled 3 hours in a di-basic phosphate solution of pH 8. The rotation of this solution by polariscope was zero.

The sugar given in Period VII was 10 gm. of pure anhydrous dextrose dissolved in distilled water. Sugar solutions were given by stomach tube.

Period.	Time.	Total N.	Glucose by polariscope.	D: N	Glucose by reduction.	D: N	Extra sugar.	Remarks.
	1917	*gm.*	*gm.*		*gm.*		*gm.*	
I	Mar. 6, 6 p.m.–12 m., 6 hrs.	4.58	13.3	2.90	15.0	3.28		
II	Mar. 7, 12 m.–6 a.m., 6 hrs.	4.01	11.0	2.75	12.7	3.17		
III	Mar. 7, 6 a.m.–12 n., 6 hrs.	3.84	11.6	3.02	12.2	3.17		
IV	Mar. 7, 12 n.–6 p.m., 6 hrs.	3.60	19.4	5.18	20.5	5.48	10.7	10 gm. of sugar phosphate mixture given at beginning of Period IV. Extra sugar in parenthesis calculated from polariscope reading.
V	Mar. 7, 6 p.m.–12 m., 6 hrs.	3.44	12.3	3.57	12.8	3.72	(10.4)	
VI	Mar. 8, 12 m.–6 a.m., 6 hrs.	3.71	11.2	3.02	12.1	3.26		
VII	Mar. 8, 6 a.m.–12 n., 6 hrs.	3.65	19.3	5.27	20.2	5.52	9.4	10 gm. of sugar given at beginning of Period VII.
VIII	Mar. 8, 12 n.–6 p.m., 6 hrs.	3.21	10.0	3.11	11.1	3.45	(8.4)	
IX	Mar. 8, 6 p.m.–12 m., 6 hrs.	3.32	10.2	3.07	11.2	3.37		
X	Mar. 9, 12 m.–6 a.m., 6 hrs.	3.48	10.0	2.87	10.8	3.09		

Average D: N of Periods II, III, and VI 3.21.
" D: N " " VI, IX, " X 3.20.

TABLE V.

Cauliflower.

Dog 5 Phloridzinized after Fasting; Weight, 14.5 Kilos.

Period	Time.	Total N.	Glucose by polariscope.	D: N	Glucose by reduction.	D: N	Extra sugar.	Remarks.
	1917	*gm.*	*gm.*		*gm.*		*gm.*	
I	Feb. 13, 6 p.m.–12 m., 6 hrs.	5.13	21.1	4.11	25.4	4.95		
II	Feb. 14, 12 m.–6 a.m., 6 hrs.	7.06	20.1	2.85	22.8	3.23		
III	Feb. 14, 6 a.m.–12 n., 6 hrs.	5.23	13.9	2.66	16.4	3.13		
IV	Feb. 14, 12 n.–12 n., Feb. 15, 24 hrs.	17.54	64.0	3.65	73.5	4.18	17.3 (15.1)	During first 6 hrs. of Period IV 500 gm. of cauliflower fed. Extra sugar in parenthesis calculated from polariscope readings.
V	Feb. 15, 12 n.–6 p.m., 6 hrs.	4.74	14.8	3.12	16.8	3.55		
VI	Feb. 15, 6 p.m.–12 m., 6 hrs.	4.21	12.7	3.02	14.2	3.37		
VII	Feb. 16, 12 m.–6 a.m., 6 hrs.	4.02	11.7	2.91	13.3	3.31		

Average D: N of Periods II, III, VI, and VII 3.21.
Available carbohydrate 3.6 per cent.

Table VI shows that from 200 gm. of cabbage 10 gm. of glucose were recovered, or 5 per cent of the weight of material fed. By diastase analysis 4.4 per cent was found. The difference is possibly within the limits of the variation in samples, or may indicate the formation of glucose from organic acids or other substances. In the case of both cabbage and cauliflower some-

what lower results were obtained from analysis than by dog method.

TABLE VI.

Cabbage.

Dog 4 Phloridzinized after Fasting; Weight, 8 Kilos.

Period.	Time.	Total N.	Glucose by reduction.	D: N	Extra sugar.	Remarks.
	1917	*gm.*	*gm.*		*gm.*	
I	Jan. 31, 12 n.–6 p.m., 6 hrs.	4.27	13.7	3.20		
II	Jan. 31, 6 p.m.–12 m., 6 hrs.	4.01	13.0	3.22		
III	Feb. 1, 12 m.–6 a.m., 6 hrs.	3.71	14.0	3.77		
IV	Feb. 1, 6 a.m.–6 a.m., Feb. 2, 24 hrs.	12.84	57.6	4.48		
V	Feb. 2, 6 a.m.–12 n., 6 hrs.	2.72	10.0	3.68	10.1	100 gm. of cabbage given at beginning of Period IV; 100 gm. 6 hrs. later. A little lard.
VI	Feb. 2, 12 n.–6 p.m., 6 hrs.	1.75	6.3	3.60		Animal exhausted.

Average D: N of Periods III and VI 3.68.
Available carbohydrate 5 per cent.

Tables VII and VIII show that nearly all the carbohydrates may be washed out of the vegetables by cooking. Snyder and coworkers[11] cooked whole heads of cabbage, determining the carbohydrate before and after cooking by the Sachasse method, and found a loss of 34 per cent of carbohydrate and fat. The

[11] Snyder, H., Frisby, A. J., and Bryant, A. P., *U. S. Dept. Agric., Bull. 43*, 1897.

TABLE VII.

*Thrice Boiled Cabbage.**
Dog 2 Phloridzinized after Fasting; Weight, 12.8 Kilos.

Period.	Time.	Total N.	Glucose by reduction.	D: N	Extra sugar.	Remarks.
	1917	*gm.*	*gm.*		*gm.*	
I	Feb. 13, 6 p.m.–12 m., 6 hrs.	3.26	14.8	4.54		
II	Feb. 14, 12 m.–6 a.m., 6 hrs.	3.92	16.0	4.08		
III	Feb. 14, 6 a.m.–12 n., 6 hrs.	3.64	14.0	3.84		
IV	Feb. 14, 12 n.–12 n., Feb. 15, 24 hrs.	13.43	50.0	3.72	1.7	During first 6 hrs. of Period IV 300 gm. of thrice boiled cabbage plus a little lard.
V	Feb. 15, 12 n.–6 p.m., 6 hrs.	3.30	13.5	4.08		
VI	Feb. 15, 6 p.m.–12 m., 6 hrs.	3.19	11.8	3.70		
VII	Feb. 16, 12 m.–6 a.m., 6 hrs.	3.11	11.1	3.57		

* Thrice boiled by method of Joslin.[6]
Average D: N for Periods III, VI, and VII 3.70.
Available carbohydrate 0.5 per cent.

greater losses in our analysis are due to the fine division of the sample before cooking.

Table IX shows the recovery of 5.9 gm. of glucose, or 1.2 per cent of weight, from 500 gm. of spinach. It is regretted that we have no analysis of spinach, but the accepted analysis by difference gives 2.3 per cent carbohydrate. The analysis of lettuce by taka-diastase gave 1.0 per cent carbohydrate. The per cent

as analyzed by difference is 2.2. This data although incomplete would indicate less of available carbohydrate in the green leafy vegetables than earlier analyses indicate.

TABLE VIII.

*Thrice Boiled Cauliflower.**
Dog 6 Phloridzinized after Fasting; Weight, 17 Kilos.

Period.	Time.	Total N.	Glucose by reduction	D: N	Extra sugar.	Remarks.
	1917	*gm.*	*gm.*		*gm.*	
I	Feb. 13, 6 p.m.–12 m., 6 hrs.	2.36	17.3	7.3		
II	Feb. 14, 12 m.–6 a.m., 6 hrs.	3.37	13.6	4.0		
III	Feb. 14, 6 a.m.–12 n., 6 hrs.	3.79	13.5	3.55		
IV	Feb. 14, 12 n.–12 n., Feb. 15, 24 hrs.	16.62	59.7	3.59	4.3	500 gm. of thrice boiled cauliflower given during first 3 hours of this period. A little lard added.
V	Feb. 15, 12 n.–6 p.m., 6 hrs.	3.78	12.3	3.25		
VI	Feb. 15, 6 p.m.–12 m., 6 hrs.	3.68	12.4	3.37		
VII	Feb. 16, 12 m.–6 a.m., 6 hrs.	3.46	11.0	3.18		

* Thrice boiled by method of Joslin.[6]
Average D: N of Periods III, V, VI, and VII 3.34.
Available carbohydrate 0.8 per cent.

TABLE IX.

Spinach.

Dog 1 Phloridzinized after Fasting; Weight, 17 Kilos.

Period.	Time.	Total N.	Glucose	D: N	Extra sugar.	Remarks.
	1917	*gm.*	*gm.*		*gm.*	
I	Feb. 6, 6 p.m.–12 m., 6 hrs.	3.96	14.8	3.74		
II	Feb. 7, 12 m.–6 a.m., 6 hrs.	4.21	14.0	3.32		
III	Feb. 7, 6 a.m.–12 n., 6 hrs.	4.11	13.5	3.28		
IV	Feb. 7, 12 n.–12 n., Feb. 8, 24 hrs.	16.28	57.0	3.53	5.9	100 gm. of spinach plus a little lard, given during the first 6 hours.
V	Feb. 8, 12 n.–6 p.m., 6 hrs.	3.78	14.5	3.83		
VI	Feb. 8, 6 p.m.–12 m., 6 hrs.	3.67	12.4	3.36		40 gm. of lard given at beginning of this period.
VII	Feb. 9, 12 m.–6 a.m., 6 hrs.	3.17	10.2	3.21		

Average D: N of Periods II, III, VI, and VII 3.29.
Available carbohydrate 1.2 per cent.

SUMMARY.

Vegetables usually used in low carbohydrate diets for diabetic patients were analyzed by the use of diastase and copper reduction, and by feeding to phloridzinized dogs with the following results.

Vegetable.	Available carbohydrate or glucose.		
	Taka-diastase.	Phloridzin-ized dog.	Atwater and Bryant.
	per cent	*per cent*	*per cent*
Cabbage	4.4	5.0	4.5
" thrice cooked	0.4	0.5	
Cauliflower	2.8	3.4	3.7
" thrice cooked	0.8	0.8	
Spinach		1.2	2.3
Lettuce	1.0		2.2

It is a pleasure to express my appreciation to Professor P. A. Shaffer, who suggested this work, for his constant interest and help.

ALKALINE RESERVE CAPACITY OF WHOLE BLOOD AND CARBOHYDRATE MOBILIZATION AS AFFECTED BY HEMORRHAGE.

By ARTHUR L. TATUM.

(From the Laboratory of Physiological Chemistry and Pharmacology, University of Chicago, Chicago.)

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Hemorrhage as an etiological factor in the causation of hyperglycemia has been for a number of years the subject of considerable investigation. Schenck (1) in 1894 made an extended study of the conditions under which hemorrhage may produce hyperglycemia. He found that when hemorrhage was produced after ligation of the liver vessels there resulted instead of an increase of blood sugar, an actual decrease, thus pointing to the liver as the source of the increased sugar, and when hemorrhage produces hyperglycemia it does so by some effect directly or indirectly upon hepatic activity. Rose (2) and also Andersson (3) made studies of the hyperglycemia of hemorrhage but did not make any essential contribution to the theory of the mechanism of its production. Nishi (4), in 1909, eliminated the function of the adrenal glands as necessary factors since he found that hyperglycemia was still produced in adrenalectomized animals following hemorrhage. Scott (5) accepted these results though admitting that emotional states may under certain conditions be a factor.

The work up to the present, so far as I am aware, does not provide a satisfactory explanation of the cause of the outpouring of sugar after hemorrhage. There might be considered emotion, changes in proportion of plasma to cells, which in itself should give an apparent rather than a real hyperglycemia, and lastly specific blood changes owing to replacement of a certain volume of blood by tissue lymph or to lack of blood volume.

In attempting to verify the generally accepted statement that hemorrhage is causative of hyperglycemia I obtained a series

of results which at first appeared inconsistent. In a series of about twenty experiments, only about half resulted in an increase in blood sugar and also in plasma sugar. The amount of hemorrhage was of about the same order of magnitude, or so nearly so that no consistent relationship appeared evident between body weight, amount of blood loss, and production of increase in sugar. In other words, the occurrence of hyperglycemia did not appear to be dependent solely upon overreaching a given volume of blood loss per body weight, but upon some controlling factor being more affected in some instances than in others. Thus it might be that the quantity of blood loss was near the limiting threshold of tolerance, such that only in a portion of the whole series of animals did there occur hyperglycemia. The rest of the series might logically be expected to have developed hyperglycemia with a larger blood loss.

Having in mind the facts recently emphasized by Murlin and Sweet (6) and by McDanell and Underhill (7) that an increase in acids was productive of a greater susceptibility toward the production of hyperglycemia and that injections of alkalies retard or prevent the development of hyperglycemia by ether, epinephrine, and other agents, it was thought that a change in the acid-base equilibrium in the body, indicated by that in the blood, may be the immediate determining factor.

The work of this paper is the report of an effort to gain further insight into some of the factors involved in the control of sugar mobilization after hemorrhage with contributory evidence regarding a chemical controlling mechanism of sugar balance.

Methods.

Blood and Plasma Sugar.

Rabbits alone were used in this series of experiments. Most of the previous study of the effects of hemorrhage on blood sugar by earlier investigators have been made on rabbits. They do not as a rule give much or any evidence of excitement on handling, are easily bled from the ear vein without any anesthetic and without struggling, and are well supplied with hepatic glycogen. Blood was drawn from the ear by cutting into but not through the marginal ear vein. The blood was collected in small wide-mouthed bottles or centrifuge tubes containing enough potassium oxalate to prevent clotting. The subsequent hemorrhages for the purpose of

studying the effects of the loss of blood were made after a period of 1 hour, more or less, as specified in the tables.

Anesthetics were purposely avoided even at the expense of increased difficulty of withdrawing of blood. The use of an anesthetic adds complications since it is impossible to standardize anesthesia and its effects on the blood contents and on activity of body cells. Different animals respond somewhat differently to ether even though care is especially taken in regard to intensity of anesthesia and its duration. This has been often observed in study of hyperglycemia following ether anesthesia. While hyperglycemia always follows, its grade varies considerably in comparing different animals. Consequently the additional variable incurred by anesthetics was purposely avoided.

For blood sugar determinations, the method of Lewis and Benedict (8), as modified by Myers and Bailey (9), was used. The Shreiner colorimeter was used for color comparison. This instrument was found to be very satisfactory for this purpose especially after placing tubes of black paper in the inner tubes to prevent the halo of paler color coming in through the sides.

Recent investigations (10) have shown some difficulty in the use of the method of Benedict for absolute quantitative estimations of reducing substances since the amount of color produced does not appear to be strictly proportional to sugar present, requiring corrections for wide limits. Yet on the use of the method for relative rather than absolute values the method appears to be entirely adequate and possesses the great advantage of simplicity and quickness when comparative values are to be utilized.

For plasma sugar determination, the blood was centrifuged as quickly as possible after hemorrhage to avoid glycolysis. Proportions of cells to plasma were determined by use of graduated centrifuge tubes, stoppered and centrifuged a fixed length of time, or until a constant volume of cells was obtained. This reading, while not so accurate perhaps as the hematocrit or specific gravity measurements, was deemed sufficiently accurate for the purposes at hand.

In the consideration of blood sugar it is recognized that blood contains reducing substances other than sugar, but fermentation tests have demonstrated that most of the reducing substance is sugar, and while other substances may be appreciable, perhaps of more importance than has been generally recognized, the inexactness in names of substances measured does not at all invalidate the fundamental arguments of this paper.

Alkaline Reserve Capacity.

The alkali-acid status of the blood was assayed by determinations of the alkaline reserve capacity of whole blood rather than CO_2 content of whole blood, or of plasma, or the CO_2-binding capacity of plasma alone. It is to be realized that venous blood is *more* or *less* venous according to the local conditions at the moment, and that therefore venosity of venous blood is itself a variable. This would appear also a considerable item

when blood is drawn for clinical purposes from the basilic vein after the application of a constrictor to distend the vein. Consequently it would seem desirable to reduce the condition of drawn blood to a standard. This was on tentative theoretical grounds approximated by rendering venous blood arterial by allowing it to come to a state of equilibrium with alveolar air which essentially renders the venous blood arterial and furthermore to a standard degree of arterialization.

The alkaline reserve CO_2 apparatus of Van Slyke (11) was used, though the technique has been somewhat adapted. Instead of using 5 per cent sulfuric acid, a 20 per cent solution of tartaric acid was used as recommended by Henderson and Morriss (12) for their special method. This acid does not seriously precipitate proteins as does sulfuric acid and consequently the extractor is very easily cleaned after each extraction by first running in 2 or 3 cc. of 10 per cent sodium hydroxide solution for the absorption of the carbon dioxide extracted from the acid-blood mixture. Then by the use of boiled distilled water the whole apparatus is readily cleaned by two or three washings. Any emulsified mercury is run out of the apparatus to be washed and dried by cotton and filter paper.

Again, owing to the fact as explained by Van Slyke that carbon dioxide is not completely extracted by fifteen successive shakings but should be corrected for unextracted carbon dioxide or by reextraction, I have adopted the procedure of allowing the mercury and acid-blood mixture to fall to the middle or lower third of the lower side reservoir after thorough mixing in the larger upper reservoir as Van Slyke directed, then raising the side reservoir to allow the fluid to reach just the upper aperture of the lower gas-cock. This now is turned allowing mercury to rise to bring the extracted gas to atmospheric pressure. Reading is now made, then the process is repeated twice after which the readings are usually constant for any given specimen.

In this paper corrections as recommended by Van Slyke are not given, since it is not absolute amounts of gas at standard conditions of saturation (temperature and atmospheric pressure) but it is *variations* in these amounts that under the same conditions indicate sufficiently for our purpose physiological variations. Furthermore normal differences in reserve capacity of different animals appear to be between considerable limits, especially in

herbivorous animals like the rabbit, while smaller but real variations are observed in the dog and in man. This relative measure obviously would not be satisfactory in a precise determination of critical points such as the lower limiting threshold of capacity from which an animal may or may not recover.

Effect of Hemorrhage on Hyperglycemia Production.

In performing a series of hemorrhages in rabbits to determine the constancy of production of hyperglycemia after a blood loss of a fairly constant proportion of total blood volume, there

TABLE I.

Blood Sugar before and after Hemorrhage. Control Experiments.

Rabbit No.	Weight.	Sex.	Blood sugar per cc.				Cells in whole blood.		Hemorrhage per kilo.	Total hemorrhage.
			Whole blood.		Plasma.					
			Before.	After.	Before.	After.	Before.	After.		
	kg.		*mg.*	*mg.*	*mg.*	*mg.*	*per cent*	*per cent*	*cc.*	*cc.*
27	1.6		1.18	1.02	1.59	1.26	31.0	27.0	18.7	30
28	1.4		1.17	1.26	1.51	1.68	34.0	23.0	21.4	30
29	1.2		1.23	1.59	1.51	1.68	31.6	21.3	25.0	30
31	1.43	♂	1.06	1.00	1.45	1.27	43.0	35.0	15.4	22
32	1.98	♀	1.22	1.29	1.38	1.51	28.0	27.0	10.1	20
33	1.1	♀	1.07	1.46	1.27	1.46	36.0	29.0	18.1	20
34	1.5		1.33	1.37	1.46	1.37	27.0	21.0	16.6	25
35	1.9	♀	1.29	1.29	1.51	1.37	37.0	31.5	13.1	25
36	1.3	♂	1.13	1.41	1.26	1.41	35.0	29.0	11.5	15
37	1.2		1.04	1.03	1.17	1.03	34.0	28.0	16.6	20
38	1.2	♀	1.37	1.61	1.61	1.80	37.5	27.0	16.6	20
40	1.48	♀	1.22	1.22	1.96	1.33	37.5	30.0	12.1	18
41	1.5	♂	1.29	1.46	1.35	1.56	30.7	28.7	13.3	20
42	1.7	♀	1.14	1.14	1.13	1.15	32.5	27.5	12.9	22
43	3.0	♀	1.08	1.02	1.17	1.14	38.0	34.5	7.0	22

appeared great irregularity of its production. As seen in Table I, approximately half the experiments developed a rise in blood sugar concentration of a significant amount while the remainder of the series suffered no demonstrable change in sugar content. This fact leads to the conclusion that it is not the mere loss of a given volume of blood *per se* but upon some other factor affected

TABLE II.

Blood Sugar and Alkaline Reserve Capacity of Whole Blood in Hemorrhage.

Animal No.	Sex.	Weight.	Time.	Hemorrhage.*	Blood sugar per cc. of blood.	Reserve capacity.	Notes.
		kg.	*1919*	*cc.*	*mg.*		
114B40	♀	2.4	Nov. 3, 2.00 p.m.	5.0	1.08	0.59	
			3.00 "	5.0	1.08	0.63	
			4.00 "	30.0	1.37	0.62	
			4.45 "	5.0	1.29	0.56	
115B40	♀	1.9	" 4, 2.00 "	5.0	1.22	0.67	
			2.45 "	5.0	1.26	0.70	
			3.30 "	26.0	1.67	0.66	
			4.15 "	5.0	2.38	0.63	
116B40	♂	3.1	" 6, 2.20 "	5.0	1.13	0.66	
			3.00 "	5.0	1.37	0.66	
			3.45 "	30.0	1.46	0.66	
			4.30 "	5.0	1.67	0.66	
			5.15 "	30.0	1.41	0.66	
			5.45 "	5.0	1.37	0.64	
117B40	♀	2.7	" 7, 2.00 "	5.0	1.73	0.63	
			2.50 "	5.0	1.61	0.65	
			3.45 "	30.0	1.56	0.66	
			4.30 "	5.0	1.73	0.65	
			5.15 "	30.0	2.16	0.60	
			5.45 "	5.0	2.82	0.54	
58B40	♀	1.8	Aug. 13, 3.15 "	4.0	1.55	0.77	
			4.00 "	4.0	1.46	0.73	
			5.00 "	18.0	1.50	0.71	
			6.00 "	4.0	1.67	0.55	
			" 14, 3.00 "	4.0	1.37	0.68	
			" 15, 9.30 a.m.	4.0	1.46	0.65	
			11.00 "	18.5	1.41	0.64	
			2.00 p.m.	4.0	1.74	0.68	
			3.00 "	14.0	2.05	0.62	
			4.30 "	4.0	1.81	0.64	
			5.30 "	14.0	3.01	0.42	

* In Tables II, IV, and VI when the hemorrhage exceeded 5 cc. in any instance the last 5 cc. drawn were used for analysis.

TABLE II—*Concluded.*

Animal No.	Sex.	Weight.	Time.	Hemorrhage.	Blood sugar per cc. of blood	Reserve capacity.	Notes.
		kg.	*1919*	*cc.*	*mg.*		
88B40	♀	1.5	Aug. 24, 4.00 p.m.	4.0	1.16	0.63	Fasting 24 hrs. before and throughout the experiment.
			5.00 "	29.0	1.41	0.60	
			6.00 "	4.0	1.67	0.57	
			7.00 "	11.0	2.38	0.52	
			" 25, 11.00 "	4.0	1.56	0.66	
64B40	♀	1.7	" 21, 2.30 "	4.0	1.33	0.56	
			5.30 "	27.0	2.05	0.44	
			" 22, 2.30 "	4.0	1.29	0.72	
			3.30 "	4.0	1.37	0.68	
			4.30 "	16.0	1.67	0.60	
			6.00 "	14.0	3.01	0.46	
			" 23, 9.00 a.m.	5.0	1.37	0.63	
61B40	♀	1.4	Aug. 18, 2.00 p.m.	4.0	1.19	0.63	
			3.00 "	4.0	1.29	0.61	
			4.00 "	25.0	1.41	0.64	
			5.00 "	4.0	1.88	0.63	
			6.00 "	19.0	2.51	0.50	
			" 19, 2.15 "	4.0	1.14	0.68	

in some instances by the loss of blood. Extending the hemorrhage to a sufficient amount invariably gives rise to a marked hyperglycemia (Table II). A surprising feature, in a number of the experiments, is the early appearance of hyperglycemia, many times being quite marked at the termination of a hemorrhage as determined by the analysis of specimens of blood obtained at the termination of the hemorrhage lasting from 10 to 15 minutes. This has also been observed by Andersson (3). In this connection it may be stated that such early appearances of hyperglycemia did not appear to be dependent upon emotional states, at least as far as these are manifested by outward signs.

Because of the view of Murlin and Underhill that the acid-base balance in the blood, and in turn in the body cells, particularly in the liver, determines the mobilization of dextrose from its storage reservoirs of glycogen, it was anticipated that a hemorrhage sufficient to produce hyperglycemia might produce changes in the acid-base balance of demonstrable grade. Consequently, studies were made simultaneously of blood sugar and of alkaline reserve capacity of whole blood. From Table II it is seen that this is the case. Those experiments in which a rise in sugar appeared were usually those in which there occurred a fall in alkaline reserve capacity of whole blood. The fall in reserve capacity, furthermore, usually appeared as soon as there appeared a rise in sugar. Those instances in which no rise of sugar appeared usually did not present a fall in reserve capacity.

Furthermore, after 12 to 24 hours, whether the animals had or had not been kept from food before and during the experiment, the sugar content fell to normal while the reserve capacity rose to and sometimes exceeded normal for that animal. The two values appeared to bear a reciprocal relationship. Fasting to a moderate extent (with access to water) from 24 to 48 hours did not prevent a return to normal values for both sugar and reserve capacity. This is taken to mean that a compensation had taken place within the body cells perhaps by hepatic action in ammonia formation from protein metabolism. This could liberate an equivalent amount of sodium bicarbonate and restore the neutrality balance.

Although no quantitative study was made of the respiratory volume, I could in no case foretell the onset or occurrence of hyperglycemia by excitement or struggling, or hyperpnea in certain of the animals in which this occurred. In other words, hyperpnea or struggling, such as occurred in our tame, frequently handled rabbits, was not necessarily concomitant with either a demonstrable fall in reserve or rise in sugar. The conclusion is not made, however, that sufficiently prolonged excitement and hyperpnea might not of itself have produced parallel blood changes. Such a problem concerns this thesis only to the extent of not being the causative factor within these series of experiments.

Effect of Alimentary Administration of Alkalies and Acids.

So far in this investigation there appears a reciprocal relationship between the rise in blood (or plasma) sugar and the fall in alkaline reserve capacity of whole blood after hemorrhage. Which of these two factors is causative and which resultant is not directly answerable from the preceding experiments. This question is approached experimentally from two directions; first, by considering that the fall in reserve capacity of blood reflects the acid-base balance in body cells and hence represents the cause of the hyperglycemia, namely cellular acidosis, second, by considering

TABLE III.

Effect on Blood Sugar of Administration of 0.6 Gm. of $NaHCO_3$ by Stomach 2 to 3 Hrs. before Hemorrhage.

Rabbit No.	Weight.	Sex.	Blood sugar per cc. of blood.				Cells in whole blood.		Hemorrhage per kilo.	Total hemorrhage.
			Whole blood.		Plasma.		Before.	After.		
			Before.	After.	Before.	After.				
	kg.		*mg.*	*mg.*	*mg.*	*mg.*	*per cent*	*per cent*	*cc.*	*cc.*
46	1.3	♂	1.19	1.20	1.10	1.16	27.0	21.0	15.3	20
47	1.98	♀	1.26	1.33	1.46	1.41	33.3	28.2	10.1	20
48	1.98	♂	1.33	1.26	1.46	1.40	41.0	35.3	10.1	20
54	1.32	♀	1.26	1.26	1.37	1.39	35.5	30.0	15.1	20

hyperglycemia after hemorrhage to cause the fall in alkaline reserve capacity of whole blood by virtue of incomplete oxidation of metabolites.

On the basis of a fall in reserve by any internal mechanism acting as the causative factor it was thought that by increasing the potential alkali in the body animals should be less subject to hyperglycemia after hemorrhage than normal controls. Hence capsules of sodium bicarbonate were given by mouth and later the animals subjected to hemorrhage of the same order of magnitude as in the preceding series. As seen from Tables III and IV hyperglycemia was, indeed, retarded in appearance. Quite by accident, following overenthusiasm over this means of medica-

tion, it was found that large quantities of bicarbonate actually facilitate the appearance of hyperglycemia on hemorrhage. This, however, appears readily explicable on the basis of Moore's work (13), in which he observes a circulatory injury by excessive tissue alkalosis which in this instance could readily explain the rise in sugar values by hepatic congestion, particularly in conjunction with a reduction in "basal blood flow" from hemorrhage as proposed by Gesell (14).

TABLE IV.

Blood Sugar and Alkaline Reserve Capacity of Whole Blood after Alkali Administration.

Animal No.	Weight.	Sex.	Time.	Hemorrhage.	Blood sugar per cc. of blood.	Reserve capacity.	$NaHCO_3$
	kg.		*1919*	*cc.*	*mg.*		*gm.*
101B40	2.6	♀	Oct. 17, 2.45 p.m.				2.1
			4.45 "	5	1.26	0.78	
			5.30 "	5	1.33	0.85	
			6.00 "	45	1.33	0.77	
109B40	1.9	♀	" 27, 9.10 a.m.				2.1
			10.10 "	5	1.29	0.93	
			11.00 "				0.7
			11.05 "	5	1.45	0.76	
			11.45 "	29	2.51	0.72	
112B40	2.1	♀	" 28, 8.30 "				0.7
			9.10 "	5	1.13	0.69	
			10.20 "	5	1.03		
			10.45 "	30	1.05	0.74	

If the basis just proposed is logical, then a diminution of body reserve in alkali by administration of acids should render the animals more subject to hyperglycemia by hemorrhage. This line of attack requires careful control since no one doubts that acids do produce of themselves hyperglycemia when given in sufficient quantity. Consequently hemorrhage was attempted after preliminary examinations had demonstrated that sugar and reserve values were approaching normal. This eliminates the

danger of development of further hyperglycemia from absorption of acid rather than from hemorrhage itself. Tables V and VI illustrate the effects of hemorrhage after acid (KH_2PO_4) administration by mouth.

While both alkali and acid administration are coupled with danger of complicating factors, it is given as contributory evidence on this thesis.

TABLE V.

Effect on Blood Sugar of Administration of 0.6 Gm. of KH_2PO_4 2 Hrs. previous to First Hemorrhage.

Rabbit No.	Weight.	Sex.	Blood sugar per cc. of blood.				Cells in whole blood.		Hemorrhage per kilo.	Total hemorrhage.
			Whole blood.		Plasma.		Before.	After.		
			Before.	After.	Before.	After.				
	kg.		*mg.*	*mg.*	*mg.*	*mg.*	*per cent*	*per cent*	*cc.*	*cc.*
49	1.9	♀	1.46	1.61	1.74	1.88	37.0	32.6	10.5	20
50	2.6	♀	1.46	1.61	1.59	1.74	32.3	30.6	7.6	20
51a*	2.6	♀	1.19	1.20	1.37	1.39	41.5	38.0	7.6	20
51b	2.6	♀	1.46	1.50	1.50	1.65	31.0	21.0	7.6	20
53	2.2	♀	1.46	1.56	1.56	1.74	33.5	30.0	9.0	20
84a†	2.8	♀	0.98	0.94					1.0	3
84b	2.8	♀	1.05	1.05					1.0	3

* Large fat rabbit with full stomach. This animal was kept from food for 18 hrs., when the experiment was repeated as No. 51b.

† In order to answer a possible objection to this series, that the increase in blood sugar after hemorrhage may be due to the continued absorption or activity of the acid rather than to the effects of hemorrhage, Rabbit 84a was fed the 0.6 gm. of KH_2PO_4 as in the rest of the series, but differing from these in that a minimum amount of blood was withdrawn. The test was repeated after 24 hrs. fasting, similar to No. 51b. The blood sugar did not rise at either time, eliminating, at least as far as one experiment may be considered as control, the direct effects of acid alone as a possible cause of the increased sugar.

TABLE VI.

Effect on Blood Sugar and Alkaline Reserve Capacity of Whole Blood of Acid Administration.

Animal No.	Weight.	Sex.	Time.	Hemorrhage.	Blood sugar per cc. of blood.	Alkaline reserve capacity.	KH_2PO_4	Notes.
	kg.		*1919*	*cc.*	*mg.*		*gm.*	
103B40	2.1	♂	Oct. 20, 9.30 a.m.				0.8	
			10.30 "	5	1.03	0.60		
			11.30 "	5	1.08	0.45		Struggled.
			1.45 p.m.	5	0.98	0.63		
			2.40 "	30	1.25	0.56		
107B40	2.1	♀	" 23, 8.30 a.m.				0.8	
			10.00 "	5	1.03	0.54		
			10.30 "	5	1.00	0.60		
			10.50 "	28	1.13	0.59		
			11.30 "	5	1.05	0.55		
113B40	2.1	♀	" 28, 3.10 p.m.				0.8	
			4.00 "	5	1.46	0.62		
			4.45 "	5	1.46	0.59		
			5.30 "	29	1.74	0.58		

Effects of Dextrose Injections.

Taking up the problem from the point of view that the hyperglycemia is causative and the fall in reserve capacity is resultant, we performed some experiments by observing the values of blood sugar and alkaline reserve after dextrose injections. As seen from Table VII, the blood sugar was increased but with no detectable change in reserve capacity. It is not, however, inferred that by using prolonged injections and in greater quantities that an acidosis in the newer sense may not be produced (15), yet it does not seem probable from these results that the sugar increase in this investigation is the cause but is rather the result of changes of cellular reaction.

TABLE VII.

Effect on Blood Sugar and Alkaline Reserve Capacity of Whole Blood of Dextrose Injections.

Animal No.	Weight.	Sex.	Time.	Hemorrhage.	Blood sugar per cc. of blood.	Alkaline reserve.	20 per cent dextrose injected intravenously.
	kg.		*1919*	*cc.*	*mg.*		*cc.*
92B40	2.4	♀	Oct. 7, 10.30 a.m.	5	1.35	0.70	
			1.30 p.m.	5	1.46	0.65	
			2.30 "	5	1.54	0.65	
			3.00 "				5
			3.30 "	5	1.96	0.65	
			4.30 "	5	1.54	0.62	
94B40	2.5	♀	" 8, 1.20 "	5	1.46	0.68	
			2.20 "	5	1.37	0.64	
			3.00 "				5
			3.30 "	5	1.76	0.68	
			5.00 "	5	1.37	0.54	
96B40	2.0	♀	" 9, 2.00 "	5	1.33	0.70	
			3.00 "	5	1.33	0.67	
			3.40 "				6
			4.00 "	5	2.05	0.65	
			5.00 "	5	1.37	0.63	

DISCUSSION.

The results of the experiments described appear to indicate that hemorrhage produces a rise in blood sugar by changes in the state of acid-base balance in body cells, which state is fairly well reflected in corresponding changes in the general circulation. The chief seat of action is probably the liver, for this is the location of glycogen storage most readily affected. Furthermore, the observations of Schenck have shown that ligation of liver vessels prevents hyperglycemia from hemorrhage. The observations of Gesell that hemorrhage lowers alkaline reserve capacity of plasma led him to conclude this was responsible for tissue asphyxia by the diminished "basal blood flow." This tissue asphyxia is effective presumably chiefly in the liver, and hence may be restated by speaking of local changes in acid-base balance in favor of intracellular acidity, a condition generally

thought adequate to facilitate glycogenolysis. A change in hepatic blood might be far more instructively studied since it is conceivable that local changes might be masked to a considerable extent by mixing with systemic blood. Direct study of portal or hepatic artery blood *versus* hepatic vein blood does not at present appear feasible since such an investigation would necessitate extensive abdominal trauma together with anesthesia both of which factors involve serious contraindications towards direct observation.

From the observations (1) that a rise in blood sugar (also plasma sugar) occurs simultaneously with a fall in alkaline reserve capacity of whole blood, (2) that alkalies administered by mouth, which enrich the portal vein blood in alkali, retard the onset of hyperglycemia, while (3) acids by the same route increase the susceptibility to hyperglycemia on hemorrhage, and further (4) that artificial enrichment of blood in sugar by injection in small quantities does not immediately produce a fall in reserve, the conclusion seems warranted that hemorrhage produces hepatic asphyxia in such a way that acids accumulate in liver cells and there promote glycogenolysis. These conditions then lay the responsibility of the rise in sugar upon the fall in alkali in cells. This fall in alkali in cells is fairly well reflected in the state of the blood in the general circulation since all tissues are more or less subjected to the same diminution of basal blood flow, yet local hepatic changes may not necessarily be quantitatively determined by systemic blood study owing to the factor of mixture of hepatic vein blood with systemic blood. The increase in sugar would not appear to be due to concentration of sugar in the blood owing to passage of water from the circulation as emphasized by Epstein (16) in certain conditions since the proportion of plasma to whole blood or to corpuscles may be found much increased ½ to 1 hour after hemorrhage, while both the whole blood and the plasma contain an increased quantity of sugar.

The author believes that the evidences presented support the contentions of Murlin and of Underhill and their collaborators that the acid-base balance in the blood and ultimately that in the body cells, particularly the liver, is one important factor determining the state of glycogenolysis as well as glycogenesis, and that further the hyperglycemia of hemorrhage can be ration-

ally explained not by invoking obscure factors such as shock, depletion, anemia, or emotional states, but on the basis of a disturbance in normal neutrality.

SUMMARY.

1. Whole arterialized blood was used in a study of alkaline reserve capacity.

2. Hemorrhage produces, if its grade is properly chosen, a reciprocal change in both a fall in the alkaline reserve capacity of whole blood and rise in blood and plasma sugar concentration.

3. Hyperglycemia and fall in reserve capacity of whole blood frequently appear within a few minutes after hemorrhage.

4. Recovery from both the reduction in alkaline reserve capacity of whole blood and the hyperglycemia occurs promptly within a relatively few hours even in the limited fasting animal.

5. Hemorrhage produces hyperglycemia more readily when the total bodily alkaline reserve is diminished by administration of acid and less readily when proper amounts of alkali are given.

6. One effective cause of sugar changes in the blood appears to be a disturbance in the acid-base balance in tissue cells indicated by that of the circulating blood in general.

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HEMOGLOBIN.

I. OPTICAL CONSTANTS.

By WILLIAM H. WELKER and CHARLES SPENCER WILLIAMSON.

(From the Departments of Medicine, and Physiology and Physiological Chemistry, College of Medicine, University of Illinois, Chicago.)

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INTRODUCTION.

In 1873, Vierordt (1) announced the definite relation of the concentration of colored solutions to the extinction coefficients of the same, in the spectrophotometer. He expressed the relation in the formula $A = \frac{c}{a}$ in which A represents the absorption constant for the substance, c, the concentration in gm. per cc., and a, the extinction coefficient. (By later writers, E is used to indicate the extinction coefficient.)

The extinction coefficient is the reciprocal of the thickness of solutions required to reduce light of unit intensity to that of 0.1 unit intensity. This epoch-making work of Vierordt made possible a new line of investigation in connection with substances forming colored solutions. Among various substances studied was hemoglobin. Von Noorden (2), Otto (3), Lambing (4), Hüfner (5), de Saint-Martin (6), Bardachzi (7), Aron and Müller (8), Butterfield (9), and Letsche (10) made a study of hemoglobin and compared the hemoglobin from some of the different species. The results that these investigators obtained did not vary sufficiently to show that the hemoglobins differed markedly in so far as their absorption coefficients were concerned. The work of Korniloff (11), Sczelkow (12), Krüger (13), and Velichi (14) seemed to indicate that the hemoglobins were not all the same since they obtained different extinction coefficients for some of them. Sczelkow explained the differences obtained by him on the basis of differences in concentration of the hemoglobin solutions he employed.

The work of Reichert and Brown (15) gave evidence that crystal forms of the hemoglobins from various species were so different that it was possible to identify species by them. Marshall and Welker (16) discovered an improvement in the method for the preparation of hemoglobin crystals. As prepared by the older methods, the solutions of hemoglobin always contain small amounts of other colloids. These colloids were removed by treatment with aluminium hydroxide cream. This treatment yielded hemoglobin solutions in the case of nearly all species which would crystallize readily. With the difference in crystal form in mind and with a fairly easy method for obtaining crystallized hemoglobin available, the study of the absorption coefficients of the hemoglobin of various species was undertaken.

EXPERIMENTAL.

Blood was drawn into a flask and defibrinated by shaking with glass beads. The defibrinated blood was strained through cheese-cloth and then centrifugalized until the corpuscles were fairly well sedimented. The corpuscles were washed with isotonic sodium chloride solution three or four times, thus removing the major portion of the serum protein. The washed corpuscles were laked by means of ether. This was added, a few drops at a time, and the liquid agitated to insure thorough mixing, and the process continued until a clear solution was obtained. If the solution was so concentrated as to be viscid, it was diluted with water and then treated with an approximately equal volume of aluminium cream. The cream was thoroughly mixed with the solution, and the mixture was then placed on a filter. The solutions of hemoglobin thus prepared were quite clear. They were cooled to a temperature of about 0°C., and treated with absolute alcohol which had also been cooled to about 0°C. until the percentage of alcohol amounted to from 20 to 30 per cent. The percentage of alcohol required depended upon the concentration of the hemoglobin solution, and also upon the solubility of the particular hemoglobin. Solutions of hemoglobin thus treated with alcohol were allowed to stand at a temperature of a few degrees below 0°C. until crystallization was complete. The crystals were then loosened from the

sides of the flask containing the solution, by agitation of the liquid. The crystals were washed by means of 25 per cent alcohol at 0°C. by decantation or by centrifugalization in a cup (17) specially built for this purpose. It is necessary that this low temperature be maintained while the hemoglobin is being washed with the diluted alcohol. If the temperature rises appreciably above 0° the hemoglobin is changed from a crystalline substance over into an amorphous substance. Some of the crystals were roughly dried by placing them on absorbent paper, and transferred to a weighing bottle of known weight. A sample of the moist hemoglobin was weighed out, dissolved in a small quantity of 0.1 per cent sodium carbonate solution, transferred quantitatively to a standard 10 cc. volumetric flask, and diluted to the mark with distilled water. After thorough mixing, a trial dilution of this fluid was made in such a way as to make the total dilution such that, when placed in the spectrophotometer, the readings would be somewhere between 70 and 80, that is, in that portion of the curve where, according to experience, the readings were most accurate. After determining approximately what the dilution should be to bring the reading at that portion of the curve indicated, an accurate dilution was made and this placed in the spectrophotometer and the readings were taken. The figures given for the optical constants represent the mean of ten readings in each position from at least two separate and closely agreeing dilutions. At least two separate crystallizations were made from each separate species of blood, using a different animal for each crystallization.

In this investigation we used the Hüfner apparatus in which the thickness of layer of solution remains constant, and E is determined by measuring intensity of the remaining light. This is accomplished by means of an analyzing Nicol prism. It has been shown that the extinction coefficient E is equal to $-2 \log \cos \theta$ where θ is the angle of rotation just referred to. In order to obtain E, the angle of rotation θ necessary to produce equality in the upper and lower field is measured and E can then be computed. Bürker has computed a curve of coefficient E for angle of rotation from 60 to 80 degrees. Having determined the angle of rotation the values of E can be read directly from this curve. The curve used in this investigation appears

to be derived from the two published previously by Bürker (18). The experimental methods used in connection with the spectrophotometric work are fully described by Williamson (19) in a previous paper.

The balance of the hemoglobin in the weighing bottle was dried to constant weight over sulfuric acid. From the figures obtained for the moisture content of this sample, the dried weight of the sample used in the spectrophotometer was calculated. After we had determined the concentration of solution and the extinction coefficient, it was a simple matter to arrive at the

TABLE I.

Absorption Coefficients.

Animal.	A'_0	A_0
Dog	0.001191	0.001878
Ox	0.001187	0.001909
Cat	0.001137	0.001778
Chicken	0.001277	0.002002
Guinea pig	0.001180	0.001895
Rat	0.001130	0.001777
Sheep	0.001224	0.001934
Horse	0.001164	0.001846
Pig	0.001253	0.001988
Human	0.001179	0.001851

value of the absorption coefficient. Determinations were made on the hemoglobins of the dog, ox, cat, chicken, guinea pig, rat, sheep, horse, pig, and man. The results are shown in Table I.

DISCUSSION.

The figures obtained vary somewhat from those obtained by other investigators. The experimental conditions of this investigation were not precisely the same as those used by the former investigators. Our readings were made in spectral region 534.0 to 542.0 while those of Hüfner were made in the region 531.5 to 542.5 and those of Butterfield in the region 533.5 to 542.0. There were other slight differences in the conditions. Butterfield (9) has pointed out that minor differences in these conditions change the results appreciably. This may possibly explain the differences in results.

CONCLUSIONS.

From the results obtained in this experiment, it would appear that there is not sufficient difference in the absorption coefficients of the hemoglobin of various species to serve as a means of identification of the species. This finding confirms the conclusions of most of the previous investigators.

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FAT-SOLUBLE VITAMINE.*

III. THE COMPARATIVE NUTRITIVE VALUE OF WHITE AND YELLOW MAIZES.

By H. STEENBOCK and P. W. BOUTWELL.

With the Cooperation of E. G. Gross and Mariana T. Sell.

(*From the Laboratory of Agricultural Chemistry, University of Wisconsin, Madison.*)

Plate 2.

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In the preceding paper of this series (1) we emphasized the necessity of using caution when attempting to generalize in regard to the probable occurrence or non-occurrence of the fat-soluble vitamine in plant or animal tissues. In the data therein presented we indicated that tissues serving in the capacity of storage organs in the plant kingdom might nevertheless contain relatively large amounts of the fat-soluble vitamine. It was found to be an unwarranted procedure to conclude that only tissues of great activity contain large amounts of the fat-soluble vitamine as has been the tendency on the part of certain investigators in this field. In instances where no differences in biological activity were apparent nevertheless great variations in fat-soluble vitamine occurred. For instance, the carrot and the yellow sweet potato were found to contain so much of the fat-soluble vitamine that as a source of this dietary essential it was necessary to class them with leafy materials rather than with the cereal grains such as barley, wheat, oats, or even maize[1] which are acknowledged to contain relatively little of it. On the other hand, the mangel, sugar beet, red beet, parsnip, dasheen,

* Published with the permission of the Director of the Wisconsin Experiment Station.

[1] In American usage, corn is a specific term used for the designation of the grain *Zea mays*, but as in foreign English speaking countries this is not the case we have used the term maize.

rutabaga, and potato, though also admittedly storage organs, were found to be decidedly deficient in it. It was moreover emphasized that, though the conclusions in regard to the relative amounts of this vitamine occurring in the different materials that were investigated were undoubtedly correct, the values were not to be considered as absolute, as, for reasons unknown, differences in the amounts present were found to occur as indicated in the vitamine content of different samples of potatoes.

That such differences undoubtedly occurred we were impressed with, when 2 years ago we started to compare the fat-soluble vitamine content of cereal grains. From the statements prevalent in the literature at that time (2) we had received the impression that maize, as an example, was very poor in its content of this dietary constituent. We were therefore considerably surprised when in our experimental lots most of our rats on yellow maize—when this was suitably supplemented with vitamine-free protein and salts—continued to grow and ultimately reached maturity and maintained themselves in good condition with no signs of malnutrition; in fact not a single instance of xerophthalmia, which usually results secondarily from the fat-soluble vitamine deficiency, was recorded in these experimental groups. Obviously the case against maize as a fat-soluble vitamine deficient food was not so strong as the investigation of others had led us to believe.

As our data on the distribution of this dietary essential accumulated we were impressed with the fact that there appeared to be a remarkable coincidence in the occurrence of yellow plant pigments and resultant success in nutrition when all other requirements outside of the fat-soluble vitamine were known to be satisfied (3). For instance, both the carrot and sweet potato which are highly impregnated with yellow pigment were found to supplement successfully rations known to be deficient in this vitamine. Other roots not so pigmented were found impotent. Butter rich in pigment is very efficient and similarly oleo oils containing the pigment show a considerable fat-soluble vitamine content. Taking another example, we have in the case of the leafy parts of plants both the growth-promoting property and the appearance of yellow plant pigments associated, though here the yellow pigments are masked by the chlorophyll. At the present

time many such correlations have been made by us and shall later be presented in their proper connections. Suffice it to say that since these general premises have apparently justified abstract inferences in regard to the probable occurrence or absence of the fat-soluble vitamine on the color basis, it appeared probable that such correlations might be extended to that of the white and yellow maize kernels to which we shall here confine our consideration. It was recalled that at one time a fat-soluble vitamine deficiency in the ration of our stock animals was indicated and that at about this time white maize instead of yellow maize had been fed as a part of their ration. No etiological connection between the two, however, was established at this time.

In the data that follow, especially as will be seen from data graphically presented in the various charts, the surmisal of the differences in comparative dietary efficiency of white and yellow maize due to fat-soluble vitamine content in correlation with the occurrence of yellow pigments was substantiated. White maize, in every case where the experimental animals were dependent on it for their fat-soluble vitamine, produced absolute nutritional failure. On the other hand, yellow maize under similar conditions gave good and, even if not in most cases, sometimes normal results.

EXPERIMENTAL.

In general, the same experimental procedure that has almost become a standard in this laboratory in the last 2 years was used in these experiments. With a few exceptions, where the ration available imposed limitations, four young rats were kept in a group and fed as such but, as noted in the graphs, sometimes data with more than four animals are recorded. In these cases the experiments were repeated and the combined data presented in one chart. No record of the amounts of the rations consumed by the animals was kept other than that indicated by the total amounts of rations consumed which made no allowance for that wasted in the cages which often totals to a considerable quantity. While accurate consumption records are much to be desired and in many instances are even indispensable for validating certain conclusions it was felt that our general conclusions would not necessitate the additional labor which the keeping of such records

would entail, especially when on such a palatable food material as maize no critical variation in amounts consumed by the animals was to be expected.

In preparation for the ration, the maize was finely ground, and mixed with casein—which had previously been made vitamine-free by very dilute acetic acid extraction—and with salts. As maize at the level fed in the experiments carries plenty of the water-soluble vitamine for growth no supplementation with this vitamine was necessary to make growth possible when all the other dietary requirements were complied with. In the main, the experiments were confined to white and yellow maize, but in addition there was investigated a red maize carrying a white endosperm, a red maize carrying a yellow endosperm, and a variegated maize of red, yellow, white, and blue effects caused by variations in the distribution of these pigments between the pericarp, the aleurone layer, and the endosperm. The specific make-up of the rations is indicated in the various charts.

Fat-Soluble Vitamine in Yellow Maize.

The yellow maizes fed were such as were well recognized as distinct varieties grown in the corn-producing sections of the United States. They were represented by Reid's yellow dent, grown extensively in the corn belt; Murdock, grown in the northern corn belt; Golden Glow, Wisconsin No. 12, grown in central and southern Wisconsin, and an early yellow dent known as Wisconsin No. 8, grown in central and north central Wisconsin.

In Chart 1 are shown the curves of growth of nineteen young rats put on the yellow corn ration soon after weaning. As seen in the chart, in general, a very satisfactory degree of growth was observed with the rats on these different varieties of maize. It is true that there obtain some variations in the growth performance of the animals, but with the limited data available these differences are not to be associated offhand with variations in vitamine content, but rather are to be attributed to variations in the natural ability of the animal to grow irrespective of the character of the ration. It is not meant to infer that differences in vitamine content may not have obtained, but rather that these were not indicated unequivocally by the experimental technique employed. In a number of instances a poor showing in growth response or ultimate failure may require special mention.

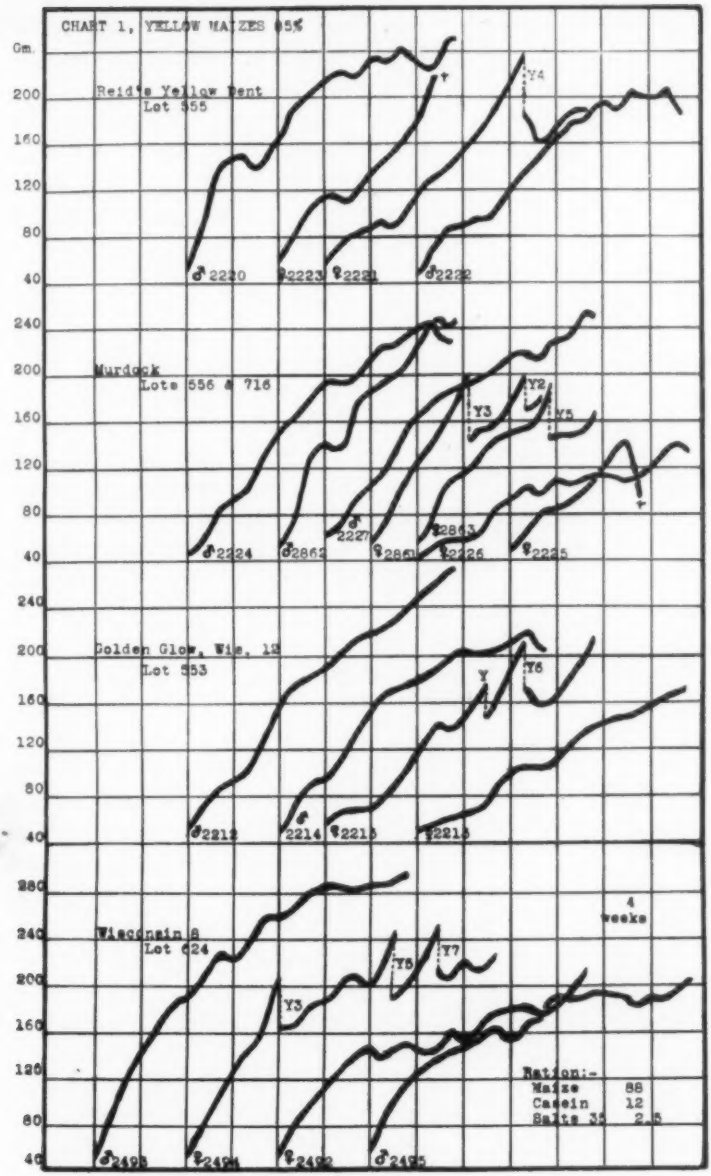
CHART 1, YELLOW MAIZES 95%
Gm.
Reid's Yellow Dent
Lot 555
Murdock
Lots 556 & 716
Golden Glow, Wis. 12
Lot 553
Wisconsin 8
Lot 624
4 weeks
Ration:-
Maize 88
Casein 12
Salts 35 2.5

CHART 1.

In Lot 555 fed Reid's yellow dent, Rat 2223 died suddenly as a result of impaction of the stomach with large pieces of shavings gathered from the litter used in the cage. Her growth had been entirely normal. In Lots 556 and 716 fed the Murdock variety of yellow maize, Rats 2225 and 2226 gave poor results. Of these, the rapid failure of Rat 2225 is to be attributed to an infection localized in the generative organs, but the cause of the subnormal growth of Rat 2226 could not be ascertained. She appeared to be in good condition except for her weight. A similar statement can be made in regard to the case of Rat 2213 of the Golden Glow group, Lot 553. In Lot 624 fed Wisconsin No. 8, no special comments are necessary except with respect to Rat 2492 which failed to expel her fetus and necessitated our chloroforming her.

One fact which stands out dominantly is that of the nine pregnancies which occurred on yellow maize not one resulted in the production or rearing of young to the weaning stage. A statement of the individual conditions may bring this out forcibly.

In Lot 555, Rat 2221 gave birth to a litter of four young which were raised to an average weight of 20 gm. in 17 days. Though slightly undersized for their age they were in good condition until one day they were found, one dead and the others very lethargic and in apparent distress. The following day all were dead. In the Murdock group, Lot 716 produced three litters of young; of these Rat 2861 nursed one individual from her first litter to a weight of 30 gm. when it died, but none of her second litter lived longer than a few days; Rat 2863 nursed her young for 10 days when they too were found missing. On Wisconsin No. 12, Lot 553, Rat 2215 came near raising her second litter. While the first litter died on the day of birth the second litter containing six individuals was nursed for 3 weeks. Normal young in our colony average about 38 gm. at this age, but these six weighed only 111 gm. and died at the end of the 4th week. Wisconsin No. 8 supported reproduction no better in Lot 624. Here Rat 2494 lost her first two litters before they were a week old and her third when slightly over 3 weeks, though it had been reduced to four animals; yet the mother remained in excellent condition.

Offhand, this failure of reproduction is not to be associated with the fat-soluble vitamine relations, but the data here presented do not give us a basis for further comment.

In Chart 1 is indicated how, over a period of about 6 months, many rats on a ration in which yellow corn served as the sole source of fat-soluble vitamine grow at a rate which is to be considered normal. That growth can be continued over a longer period of time is shown in Chart 2. The rats of this group were fed a similar ration to those whose growth curves are shown in Chart 1 differing only in that to the 88 gm. of maize and the 12 of casein 0.75 gm. of sodium chloride, 4.43 gm. of calcium lactate, and 0.14 gm. of iron citrate were added instead of the 1.0 gm. of sodium chloride and 1.5 gm. of calcium carbonate. On this ration Rat 1707′ and 1705′ grew over a period of 13 months at

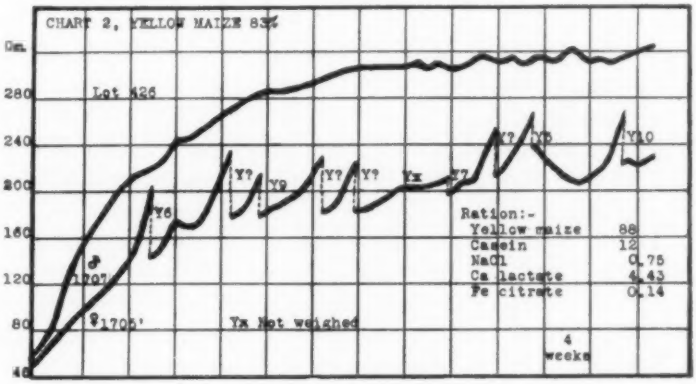

Chart 2.

rates which can be considered normal though it is probably true that the initial rapid increase of weight was not continued for so long a period as might have been expected for the average of our colony, but this may not have been entirely due to a limitation of the fat-soluble vitamine factor, as the same result has often been obtained on other supposedly good rations—including yellow maize—which we had supplemented with this vitamine. In the case of the female, no doubt her numerous pregnancies delayed her growth; yet she maintained herself in excellent condition. The male grew better, yet after the first 8 months of growth it became rather rough in appearance although there was no evidence of any cutaneous infections; its eyes remained large and bright and it continued to be sexually potent to the time

of writing. Unequivocal indications of a fat-soluble vitamine deficiency may then be said to have been totally absent.

It was noted in the discussion of the graphs in Chart 1 that absolute failure in the rearing of the young was the rule. This was duplicated in the first eight pregnancies of this female, in fact all the young were found dead shortly after birth or else were found missing before any record of their number or weight had been obtained. Our data on this are complete with one exception where by an oversight with a change in the laboratory staff the rat was not weighed or closely observed for 3 weeks, and so it is not known how long that litter lived. To provide more favorable conditions for the rearing of the young, the young of the ninth pregnancy were reduced in number to three animals shortly after birth. These were reared rapidly, averaging 36 gm. in weight at the end of 3 weeks and 45 gm. at the end of 4 weeks which is a rate of growth that can be considered normal. With the tenth litter reduced to four animals this performance was duplicated. We have therefore secured information showing that it is possible to secure the rearing of some young on a ration where yellow maize is the sole source of the fat-soluble vitamine. In instances where lactating mothers were transferred when mature from our standard stock ration to the corn ration fed in this lot, no difficulty in the rearing of young has been experienced, in fact, both the experimental Rats 1707′ and 1705′, whose history was just discussed, were members of a litter brought up under these conditions. It is thus indicated that, when rats make their growth on such a ration and have later the added demands of lactation imposed on them, they cannot meet the requirements of the situation as they can when they have been reared on a better ration. But it is not to be inferred that the fat-soluble vitamine is necessarily the limiting factor. Furthermore, it remains to be seen if the fact that the young of the earlier pregnancies were not reared, while those of the later were, has any special significance.

Fat-Soluble Vitamine Content of White Maizes.

When young rats at an entirely comparable period in their development to those shown in Charts 1 and 2 are put on white maize supplemented with purified proteins and salts, failure in

growth and even in maintenance of life rapidly ensues. This is shown in Chart 3. In the dissection of these data we are limited in the conclusions that we can draw by the fact that differences in growth performances can, to a certain extent—not determined—be attributed to variations in the growth impulse of the animals. To a very great degree these differences should be erased by the method we used in the selection of our animals, because in this procedure animals are selected for differ-

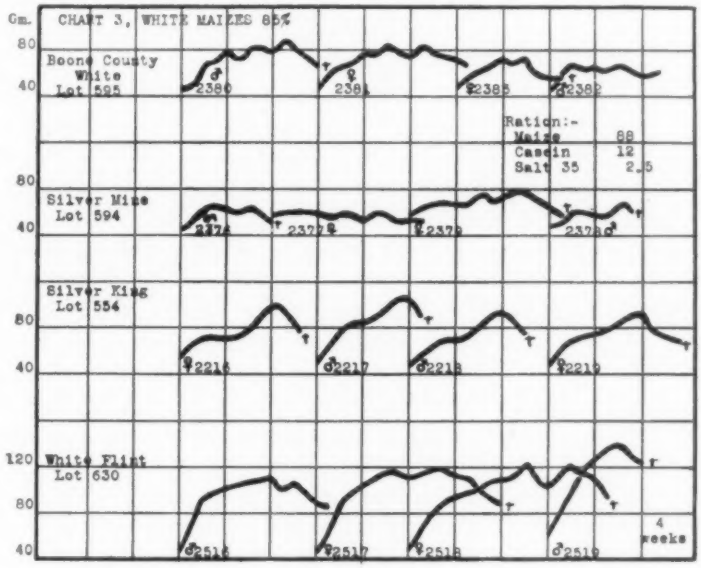

CHART 3.

ences in coat patterns from a group of 50 to 100 of suitable age and size gathered in one cage. In this way the chances are that few if any groups of four consisted of individuals from only one litter and therefore they would be unlikely to have similar inherited tendencies. With this in mind it appears rather remarkable that there should obtain such similarity in the character of the curves of growth of the animals within the groups and such distinguishing features—at least in the amount of growth—among the different groups if differences in nutritive value did not obtain. We are inclined to believe that the better

growth performance of the White Flint group was due to a greater fat-soluble vitamine content. This is noteworthy especially as the White Flint maize though a commercial white corn was not a "dead" white in comparison with the Silver Mine variety. Some allowance must be made for the difference in appearance bound to result in a flint maize as compared with the dent, yet making this allowance this variety appeared to have a distinct tinge of yellow both in the grain and as a meal. We do not care to stress this point reserving our conclusions until appearances are verified by chemical examination, but with the evidence given by the growth curves we believe it highly probable that there are differences in the vitamine content of the so called white maizes corresponding to their degree of yellow pigmentation. Certain it is that all these varieties are far different in their fat-soluble vitamine content from those of the yellow variety.

In the four lots receiving white maize as the sole source of the fat-soluble vitamine in their ration we had abundant demonstration of its deficiency in this constituent by the observed symptom complex which while not entirely specific yet in a large group of animals gives indications of the exact nutritive status with a fair degree of certainty. First in importance there is the failure of growth ending in failure of maintenance of life; second there is the often occurring inflammation of the eyes, a conjunctivitis or xerophthalmia as pointed out by Osborne and Mendel; and thirdly there may prevail the general condition of malnutrition of the skin as indicated by encrustation of the ears, warts on the nose, infection on the tail and feet, and even sores on the body itself. All these occur singly or collectively, but as they are not absolutely specific they are to be considered suggestive symptoms instead. In the group of rats all failed to grow and no doubt all would have died if the experiments had been continued a short time longer as twelve of the sixteen animals died during the time of observation. Of the total, nine had contracted an infection in one or both eyes and three had cutaneous infections.

Comparative Fat-Soluble Vitamine Content of White and Yellow Maizes.

In order to bring out the limitations in the amounts of fat-soluble vitamine in yellow and white maize more concretely than the previous lots had indicated two experimental groups were fed these grains in which 50 instead of 88 parts of the ration—exclusive of the added salts—consisted of maize (Chart 4). The results are obvious. They indicate that with a reduction in the amount of white maize in the ration growth is little worse than with some of the white maizes at the higher level. Unfortunately the white maize fed in this lot was not of any standard variety.

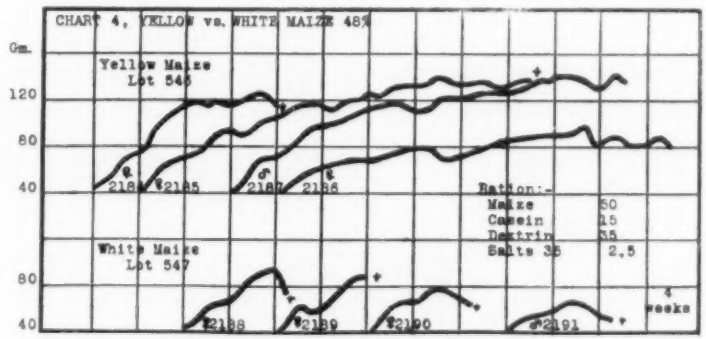

CHART 4.

but was obtained on the local market as a distinctly white variety so direct comparisons are not possible, but apparently it seems safe to infer that certain white maizes contain so little of the fat-soluble vitamine that as a source of this dietary essential they can be considered valueless. This is the only conclusion that can be arrived at when it is seen that a reduction in the amount of white maize in the ration causes so little difference in the resultant growth. Such growth as is observed must be attributed to the fat-soluble vitamine stored in the body of the animal. Three of the animals, *viz.* Rats 2188, 2190, and 2191, developed xerophthalmia, but Rat 2189 died without any visible signs of infection.

With the yellow maize a reduction of the amount in the ration from 85 to 48 per cent gives a definite corresponding reduction in the amount of growth. Growth was possible at only about one-

half the normal rate from which we may infer—as the reduction in maize and therefore vitamine content was approximately 40 per cent—that yellow maize when fed in maximum amounts contains just sufficient fat-soluble vitamine to make normal growth possible.

Fat-Soluble Vitamine Content of Red Maizes.

With a relation between the occurrence of the yellow pigment and the growth-promoting property associated with the fat-soluble vitamine established in the case of yellow maize, it re-

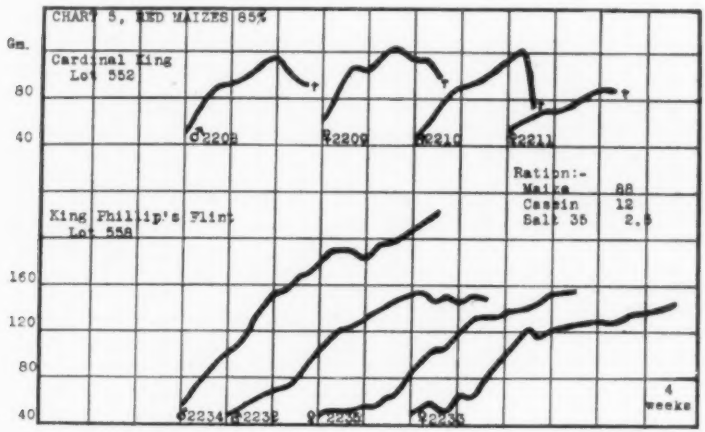

CHART 5.

mained to determine what the situation might be in the case of maizes of other colors. This opportunity presented itself with two varieties known respectively as Cardinal King and King Phillip's Flint. It is seen at a glance in Chart 5 that failure on the former ensued rapidly while on the latter growth continued at a subnormal rate for 22 weeks when the experiment was terminated. The difference can again be correlated with reference to the presence of yellow pigments for while both varieties were red, the Cardinal King had a white endosperm while the King Phillip's Flint had a yellow endosperm; the former has no visible yellow pigments, while the latter has considerable.

The rats on Cardinal King in Lot 552 succumbed to the fat-soluble vitamine deficiency very rapidly, all dying in less than 12 weeks after they had been put on the ration. Their behavior was very similar to those on the white maize, Chart 3. All except Rat 2211 contracted xerophthalmia.

On King Phillip's Flint when the experiment was discontinued —almost 23 weeks after its inauguration—all the animals were rated as being in good condition giving no evidence of eye infections or cutaneous malnutrition.

Fat-Soluble Vitamine Content of Variegated Maize.

Maize, as indicated by the innumerable so called varieties which are grown in the United States, offers many opportunities for the intermingling of characters not only in habit of growth, but also in color. In variegated maize many color characteristics

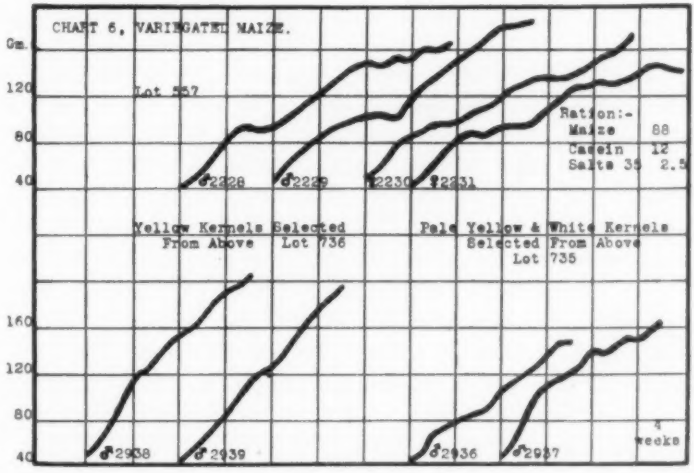

CHART 6.

may be associated in the same kernel or in different kernels on the same cob. Such a maize was the sample whose fat-soluble vitamine content was demonstrated in Lot 557, Chart 6. All the rats grew a little better than one-half the normal rate and were in good condition when the experiment was discontinued.

It is noteworthy that there again the maize fed had a very large proportion of its kernels bearing varying amounts of yellow pigment especially in the endosperm, but some of the kernels were distinctly white and others, when pigmented, had a white endosperm.

It remained to establish how closely the fat-soluble vitamine was associated with the yellow pigments in this sample of maize where color characteristics were so closely interwoven that from the hereditary standpoint it was impossible to predict what the color distribution in the crop from any one kernel would be. To determine this, the seeds predominatingly yellow were selected by hand from those pale yellow or more nearly white. The results are shown in Lots 735 and 736. As before, the intimate association of yellow pigment and the property attributed to the occurrence of the fat-soluble vitamine obtains. We believe all our data substantiate this thesis beyond a doubt.

Supplementation of White Maize with Fat-Soluble Vitamine.

As all our rations, outside of the fat-soluble vitamine, contained all the generally recognized required ingredients of a complete ration and as many of our animals on the white maize rations consistently, singly or collectively, gave all the symptoms of a fat-soluble vitamine deficiency there remains no alternative but to conclude that the failure of rats to grow on white maize rations is due to a lack of fat-soluble vitamine. It seems rather remarkable that these differences in the nutritive value of the maizes should not have been generally recognized especially when it is considered that both are used in large amounts for feeding animals. As a matter of fact it has been reported by some stockmen that yellow maize is superior to white maize, but again by others it has been as stoutly denied. As chemical analysis for determining nutritive values of feeds has indicated no differences in digestible fat, protein, nitrogen-free extract, fiber, and ash, the entire matter has been given little attention in nutrition fields, as the limitations of such analysis have been little realized (4). Chart 7 indicates, if data obtained with rats are applicable to other animals, that from the practical standpoint this difference of opinion may be based on justifiable grounds. Ordi-

narily materials rich in the fat-soluble vitamine are consumed by man, as well as by animals, in sufficient amounts to supplement the fat-soluble vitamine-poor foods efficiently. But under restricted feeding conditions the situation is far different. It ap-

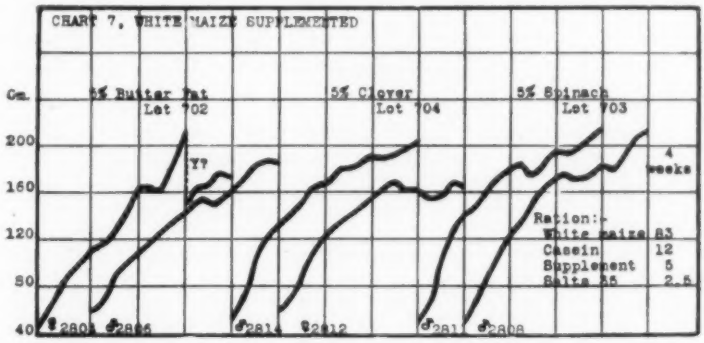

CHART 7.

pears a curious reflection on human instinct that in the United States and Mexico white maize is preferred to yellow maize as a major constituent of the diet, especially when it is considered that this is the case in sections where restricted rations are common.

CONCLUSIONS.

The occurrence of yellow pigment and the growth-promoting property attributed to the presence of the fat-soluble vitamine seem to be intimately associated in the maize kernel.

Yellow maize contains enough of the fat-soluble vitamine to allow growth at the normal rate to take place in the rat; reproduction was possible, but usually was a failure; maintenance without premature signs of senility was also observed.

White maize does not contain any demonstrable amounts of the fat-soluble vitamine. In attempts to have it serve as the source of fat-soluble vitamine it seldom allows rats to remain alive longer than 3 months. One commercially so called white maize allowed a certain amount of growth to take place, but close inspection indicated the presence of some yellow pigment.

Red maize with a white endosperm free from yellow pigment gave the same results as white maize; red maize with a yellow

endosperm gave results though not of the same degree, yet approximately that of yellow maize.

A variegated maize of red, yellow, white, and blue effects due to variation in color distribution in different kernels between pericarp, aleurone layer, and endosperm gave results intermediate between those obtained with yellow and white maize; in correlation most of the kernels had yellow endosperms, the rest had white endosperms.

When from the variegated maize the yellow kernels were selected from those that were white or a very pale yellow and fed to young rats, the performance of growth was distinctly better on the former.

In a mixed diet containing maize the fat-soluble vitamine deficiency of white maize is easily taken care of by the supplementary action of other foods rich in this dietary essential.

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EXPLANATION OF PLATE 2.

FIG. 1. Rat 2215, female, after having been fed for 78 days on a ration in which all the fat-soluble vitamine was supplied by 85 per cent of yellow maize. When started on the ration it weighed 59 gm.; when photographed 137 gm. Note its good nutritive condition. For weight record see Chart 1, Lot 553.

FIG. 2. White Rat 2377, female, and hooded Rat 2379, female, after having been fed for 92 days on a ration in which all the fat-soluble vitamine was supplied by 85 per cent of white maize. For weight records see Chart 3, Lot 594. The white individual originally weighed 47 gm.; when photographed it weighed 53 gm. Its ears were infected, but its eyes were normal.

The hooded rat originally weighed 57 gm.; when photographed it weighed 60 gm. Note the encrustation of the ears and the inflamed eye. It died 2 days later.

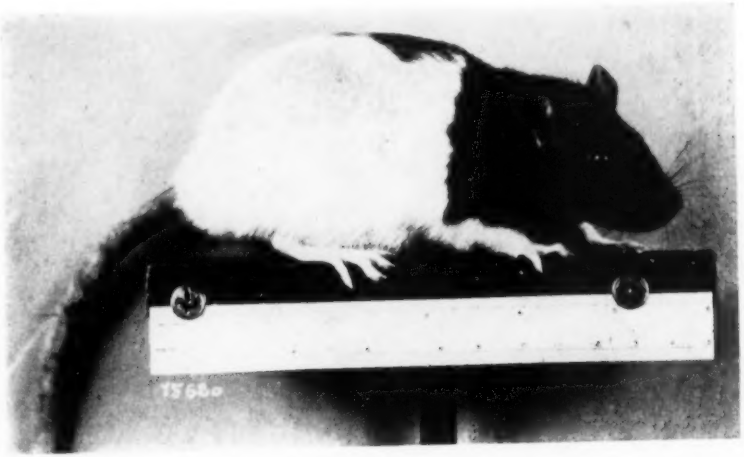

FIG. 1.

FIG. 2.

PROTEIN REQUIREMENT OF MAINTENANCE IN MAN AND THE NUTRITIVE EFFICIENCY OF BREAD PROTEIN.

By H. C. SHERMAN.

With the Cooperation of L. H. Gillett and Emil Osterberg.

(*From the Department of Chemistry, Columbia University, New York.*)

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In connection with our studies of nutritive requirements and of the efficiency of the proteins of the cereal grains in the maintenance metabolism of man[1] we have taken occasion to bring together the results of such of the earlier experiments upon the protein requirement of maintenance as seem to lend themselves to direct quantitative comparison. The purpose of the present paper is to present as concisely as possible the net result of this study of the literature together with the data of our own experiments upon a dietary in which nearly all the nitrogen was taken in the form of wheat bread.

An attempt to determine the protein requirement of maintenance, even for any one kind of protein, would ideally involve adherence to an otherwise uniform diet with complete findings of nitrogen intake and output and with periodical increase or decrease of protein consumption without alteration of the energy supply, until it is shown that the body can maintain itself in nitrogen equilibrium on a certain amount of protein, of the kind or kinds which the diet in question furnishes, and not on any smaller amount. The recorded investigations which meet all these conditions are not sufficiently numerous, nor do they cover a sufficient number of men and women or a sufficient range of foods to form a satisfactory basis for any general deduction as to

[1] Sherman, H. C., Gillett, L. H., and Pope, H. M., *J. Biol. Chem.*, 1918, xxxiv, 373. Sherman, H. C., Wheeler, L., and Yates, A. B., *ibid.*, 1918, xxxiv, 383. Sherman, H. C., and Winters, J. C., *ibid.*, 1918, xxxv, 307. Sherman, H. C., Winters, J. C., and Phillips, V., *ibid.*, 1919, xxxix, 53.

the amount of protein required in normal adult maintenance. In attempting such a deduction, proper consideration must also be given to the much larger number of experiments in which the balance of intake and output of nitrogen has been determined with subjects living on low protein diets under conditions so arranged and controlled as to make it probable that the rate of protein metabolism was at least approximating the minimum at which normal equilibrium could be maintained.

Probably the best present indication of the normal protein or nitrogen requirement is to be obtained by averaging the observed nitrogen output in all available experiments in which the intake appears to have been barely sufficient or not quite sufficient to result in equilibrium of intake and output.

Since the protein minimum thus determined is influenced by the protein-sparing action of carbohydrates and fats, the results will be comparable, and will bear directly upon the practical problems of protein requirement in food economics, only in those cases in which the energy value of the experimental ration is at least approximately adjusted to the energy requirement of the subject.

Many of the published experiments which were designed to test the amounts of protein required in normal nutrition are now seen to have given misleadingly high results either because the food was reduced as a whole, thus creating a deficit in the energy intake which interfered with the economical use of the protein, or because, influenced by past overestimates of the protein requirement, the experimenters did not sufficiently reduce the amount of protein in the food to make a real test of the minimum on which equilibrium could be established and maintained.

Given a proper adjustment of the energy value and a sufficiently restricted proportion of protein in the experimental ration, the question arises as to how closely the nitrogen output must agree with the intake in order that it may be accepted as indicating the approximate requirement of the subject under the conditions of the experiment.

A plus balance or exact equilibrium of nitrogen intake and output always suggests the question whether equilibrium might not have been again established if the nitrogen intake had been further reduced, in which case the given result was obviously higher than the amount actually required. Whether the observed

output is such an overestimate or is a fair approximation to the amount really required for equilibrium can often be judged with a fair degree of confidence from a detailed and critical study of the investigation as a whole. At any rate, when there is nitrogen equilibrium on a low protein intake it seems safe to conclude that the diet is at least meeting all the requirements of the protein metabolism of maintenance.

Also, when the nitrogen output is only slightly greater than the intake it seems permissible to regard the output as approximating the amount on which equilibrium could have been maintained; for while it is conceivable that a small loss of body nitrogen may represent a real inadequacy of the intake, perhaps as regards some particular amino-acid, yet it is usually much more probable that a small negative balance means simply that the body has not yet completed the adjustment of its output to its intake and that a continuation of the experiment would have shown a lower output. Thus while it is possible that the output may sometimes be smaller than the amount which would be required for equilibrium, yet from the large amount of evidence now available it seems fairly certain that any error of this sort can have but very slight influence upon the final average, and in all probability is more than offset by the tendency toward high results introduced through including in the average a considerable number of experiments in which the protein of the diet was not reduced to a sufficient extent, and for a long enough time, to show the real minimum at which normal equilibrium could be maintained.

In order to minimize the personal equation in our interpretation of the work of others, we have uniformly excluded from Table I all experiments showing a loss of body nitrogen greater than 1 gm. per day even though in some cases this necessitated the omission of data of undoubted value.

There remained 109 experiments belonging to twenty-five different investigations and including 67 experiments upon twenty-nine men and forty-two experiments upon eight women subjects. For convenience of comparison the total nitrogen output per day (urine and feces) is computed to a basis of 70 kilos of body weight and multiplied by 6.25 to express the corresponding amount of protein which is tabulated as the "indicated protein requirement" in each of the 109 experiments as shown in Table I.

TABLE I.*

Indicated Protein Requirements per 70 Kilos of Body Weight.

Experiment No.	Indicated protein requirement.	Experiment No.	Indicated protein requirement.	Experiment No.	Indicated protein requirement.	Experiment No.	Indicated protein requirement.	Experiment No.	Indicated protein requirement.
	gm.		gm.		gm.		gm.		gm.
1	39	23	45	45	31	67	56	89	37
2	38	24	49	46	37	68	57	90	38
3	45	25	56	47	45	69	60	91	39
4	33	26	47	48	50	70	57	92	39
5	30	27	62	49	39	71	54	93	39
6	65	28	61	50	52	72	51	94	43
7	61	29	55	51	52	73	52	95	40
8	41	30	53	52	42	74	54	96	39
9	50	31	53	53	42	75	54	97	43
10	43	32	61	54	24	76	53	98	38
11	37	33	40	55	21	77	53	99	37
12	29	34	56	56	33	78	44	100	33
13	59	35	43	57	32	79	45	101	37
14	57	36	42	58	32	80	46	102	41
15	49	37	41	59	39	81	46	103	51
16	42	38	39	60	50	82	42	104	44
17	50	39	48	61	58	83	41	105	42
18	48	40	46	62	37	84	36	106	32
19	49	41	38	63	41	85	38	107	35
20	54	42	47	64	44	86	39	108	34
21	36	43	36	65	59	87	39	109	32
22	47	44	37	66	60	88	38		
Average									44.4

* Experiments 1 and 2, Hirschfeld, F., *Arch. ges. Physiol.*, 1887, xli, 533. No. 3, Hirschfeld, F., *Virchows Arch. path. Anat.*, 1888, cxiv, 301. Nos. 4 and 5, Klemperer, G., *Z. klin. Med.*, 1889, xvi, 550. Nos. 6 and 7, Viot, C., *Z. Biol.*, 1889, xxv, 232. No. 8, Pechsel, Eiweissbedarf des gesunden Mensch., Dissertation, Berlin, 1890 (*U. S. Dept. Agric., Bull. 45*, 1898). No. 9, Lapicque, L., *Arch. Physiol.*, 1894, Series 5, vi, 596. Nos. 10 and 11, Sivén, V. O., *Skand. Arch. Physiol.*, 1900, x, 91. No. 12, Sivén, V. O., *ibid.*, 1901, xi, 308. No. 13, Albu, *Z. klin. Med.*, 1901, xliii, 75. Nos. 14 to 20, Jaffe, M. E., *U. S. Dept. Agric. Bull., 132*, 1902. No. 21, Caspari, W., and Glaessner, K., *Z. diät. physik. Therap.*, 1903, vii, 475. Nos. 22 to 33, Chittenden, R. H., Physiological economy in nutrition, New York, 1904. No. 34, Aron, H., and Hocson, F., *Biochem. Z.*, 1911, xxxii, 189. Nos. 35 to 38, Hindhede, M., *Skand. Arch. Physiol.*, 1912, xxvii, 277; Nos. 39 to 46, 1913, xxx, 97; Nos. 47 to 55, 1914, xxxi, 259. Nos. 56 to 61, Abderhalden, E.,

The 67 experiments upon men show an average "indicated protein requirement" of 0.633 gm. per kilo, while the 42 experiments with women average 0.637 gm. per kilo of body weight. It seems unnecessary, therefore, to distinguish between the sexes in this discussion since we are dealing with data calculated to a uniform basis of body weight.

The general average of the 109 experiments shows an indicated requirement of 0.635 gm. of protein per kilo of body weight, or 44.4 gm. per "average man" of 70 kilos, per day. Two considerations, one favorable the other unfavorable, should be kept in mind in attempting to judge the scientific value of this average. On the one hand, it represents a very large amount of work in several different laboratories and on many subjects, both men and women, so that errors due to individual peculiarities of subjects, diets or conditions, or the personal equation of the investigator are minimized. On the other hand, the data of individual experiments are rather divergent, ranging from a minimum of 21 gm. to a maximum of 65 gm. per 70 kilos. It should perhaps be noted that the very large majority of these experiments (94 in 109) yield values within the limits of 29 to 56 gm. per 70 kilos, and that these more concordant data taken separately show an indicated protein requirement averaging 42.8 gm. per 70 kilos based on 94 experiments covering thirty-four subjects (twenty-six men and eight women) studied in twenty-three different investigations. If we go a step further in rejecting the extremes, we find within the limits of 30 to 50 gm. per 70 kilos per day, 76 experiments belonging to nineteen different investigations, including twenty-four subjects (twenty men and four women) and averaging 40.6 gm. per man of 70 kilos, or 0.58 gm. per kilo of body weight per day.

Ewald, G., Fodor, A., Röse, C., *Arch. ges. Physiol.*, 1914–15, clx, 511. Nos. 62 to 64, Rose, M. S., and Cooper, L. E., *J. Biol. Chem.*, 1917, xxx, 201. Nos. 65 to 72, Sherman, H. C., Gillett, L. H., and Pope, H. M., *ibid.*, 1918, xxxiv, 373. Nos. 73 to 77, Sherman, H. C., Wheeler, L., and Yates, A. B., *ibid.*, 1918, xxxiv, 383. Nos. 78 to 93, Sherman, H. C., and Winters, J. C., *ibid.*, 1918, xxxv, 301. Nos. 94 to 102, Sherman, H. C., Winters, J. C., and Phillips, V., *ibid.*, 1919, xxxix, 53. Nos. 103 and 104, Sherman, H. C., and Rose, A. R., not previously published. No. 105, Sherman, H. C., and Beegle, F. M., not previously published. Nos. 106 to 109, Sherman, H. C., and Osterberg, E., not previously published.

Thus according as we include all 109, or 94, or 76 experiments in the average it becomes respectively 0.635, 0.61, or 0.58 gm. per kilo, or in round numbers 45, 43, or 41 gm. per man of 70 kilos per day. It will be noted that the more critical the selection of the experiments to be included in the average the lower the indicated protein requirement becomes. There would appear to be a smaller probable error in estimating the average protein requirement at about 0.6 gm. per kilo, or 42 gm. per 70 kilos, per day, than at any higher figure. It should also be noted that the final data of nearly all the more extended and the more closely controlled investigations fall below rather than above this average.

From a general review of all the experiments included in this compilation it appears to us that the chief cause of variation was the differing duration of the investigations and the differing extent to which the subject had accustomed himself to a low protein diet. In the work thus far published this seems to have been a more influential factor in determining the amount of protein apparently required for maintenance in the human adult than was the nature of the protein fed.

We have previously shown that the protein of rations consisting essentially of corn (maize) meal or oatmeal supplemented by only small amounts of milk, the latter furnishing but one-tenth to one-fifth of the food protein, may be fully as efficient in the maintenance metabolism of man as the average protein of ordinary mixed diets has been found to be in the work of previous investigators. Because of the large extent to which the majority of the people of this country and of Europe depend upon bread as their chief source of protein as well as energy, it seems well to give in this connection the data of our chief series of experiments in which ordinary (white) wheat bread furnished almost all the protein of the food.

Experiments upon the Efficiency of Bread Protein in Maintenance Metabolism.—The subject, E. O., a man weighing 80 kilos, took on each of 15 successive days a ration consisting of 400 gm. of bread, 150 (or, during the last 6 days, 200) gm. of butter, and 300 gm. of apple. The 15 days were divided into five experimental periods of 3 days each, the foods and feces being collected, sampled, and analyzed separately for each of these periods. Urine was collected and analyzed separately for each 24 hours. The work

thus constitutes a series of five complete balance experiments each of 3 days duration and following each other without intermission. The data of nitrogen intake and output are shown in Tables II and III.

In these experiments bread furnished over 95 per cent of the protein consumed, yet allowing the first 3 days for adjustment it will be seen that practical equilibrium was maintained on an intake of a little less than 0.5 gm. of protein per kilo of body weight per day. Thus the protein of wheat bread showed as high an efficiency in the maintenance metabolism of man as would be expected of the protein of mixed diet in general. The bread was ordinary white bread purchased from a New York City

TABLE II.

Food Eaten with Daily Intake of Nitrogen from Each Source. Experiments with E. O., Weight 80 Kilos.

Kind of food.	Experiment 1.	Experiment 2.	Experiment 3.	Experiment 4.	Experiment 5.
	gm.	*gm.*	*gm.*	*gm.*	*gm.*
Bread	400	400	400	400	400
Butter	150	150	150	200	200
Apple	300	300	300	300	300
Nitrogen from bread	5.77	5.87	5.76	5.71	5.72
" " butter	0.13	0.13	0.13	0.18	0.18
" " apple	0.11	0.09	0.11	0.11	0.12
Total per day	6.01	6.09	6.00	6.00	6.02
Protein per day	38	38	38	38	38

bakery. Probably the customary small amount of milk was used in making the bread—exactly how much or in what form we were not permitted to ascertain. That the bread did not contain any unusual proportion of milk or milk powder was shown by determining its content of phosphorus and calcium as well as of nitrogen.

Thus it does not seem necessary to discriminate against bread protein as compared with the protein of staple foods in general in so far as the requirements of adult maintenance alone are concerned. This result is in opposition to the claims of Karl Thomas but is in accordance with the findings of Hindhede and of Morgan

and Hintze in their experiments upon man, as well as with the work of this laboratory on the efficiency of maize and oat proteins in adult human nutrition, and is in harmony with the findings both of Osborne and Mendel and of McCollum and his

TABLE III.

Daily Intake and Output of Nitrogen. Experiments with E. O.

Experiment No.	Day.	Body weight.	Nitrogen.				
			In food.	In urine.	In feces.	Output.	Balance.
		kg.	*gm.*	*gm.*	*gm.*	*gm.*	
1	1st	81.0	6.0	6.6			
	2nd		6.0	6.6			
	3rd		6.0	6.6			
Average			6.0	6.6	0.7	7.3	−1.3
2	4th	80.5	6.1	5.5			
	5th		6.1	4.3			
	6th		6.1	5.1			
Average			6.1	5.0	0.9	5.9	+0.2
3	7th	80.5	6.0	5.2			
	8th		6.0	5.4			
	9th		6.0	5.7			
Average			6.0	5.4	1.0	6.4	−0.4
4	10th	80.5	6.0	6.0			
	11th		6.0	4.5			
	12th		6.0	5.2			
Average			6.0	5.2	1.0	6.2	−0.2
5	13th	80.3	6.0	4.5			
	14th		6.0	5.1			
	15th	79.8	6.0	4.7			
Average			6.0	4.8	1.0	5.8	+0.2

associates that the proteins of cereal grains need only be fed in very moderate quantity to provide for the maintenance of body weight in adult rats. Thus Osborne and Mendel found the body weight maintained by food mixtures which contained only

6.5 to 7 per cent of the proteins of the whole wheat kernel,[2] which, as these food mixtures contained about 25 per cent fat, would mean that wheat protein sufficient to furnish from 5 to 6 per cent of the total calories of the food consumed was sufficient for maintenance. McCollum[3] gives 4.5 per cent of oat protein or 6 per cent of wheat or maize protein as the proportion necessary to maintain body weight in adult rats. In McCollum's food mixtures the percentage of protein by weight would be about the percentage of protein calories in the total calories of the food.

Thus the feeding experiments upon rats are fairly consistent in indicating that the amount of grain protein required for adult maintenance (in this case judged by body weight) is about 6 per cent of the total food calories. Turning to the experiments upon the maintenance of nitrogen equilibrium in man, we see from the data above cited an indicated average protein requirement of about 0.6 gm. of protein per kilo of body weight per day. This corresponds to 2.4 protein calories, or 6 per cent of the 40 calories per kilo which is commonly accepted as a fair average energy requirement for moderately active men and women. Thus it would appear that the protein minimum for normal adult maintenance is very similar for man and the rat if the protein is stated in terms of the total calories of food consumed. It does not necessarily follow that the protein requirement of growth will be proportionately as high for the child as for the young rat. The percentage rate of growth is very much greater in the young rat than in the child and healthy children are usually more active than the average of young rats at corresponding stages of growth. From these facts it is to be expected that the requirement for protein relative to calories will be greater in the case of the young rat than of the child at a corresponding stage of development. Relative to body weight, the protein requirement of growth is of course higher than that of maintenance, in either species. Of at least equal importance is the fact that the amino-acid make-up of the food protein is a more prominent factor in the problem of growth than in that of maintenance. In connection with the well known work of Hopkins, of Osborne and Mendel, and of

[2] Osborne, T. B., and Mendel, L. B., *J. Biol. Chem.*, 1919, xxxvii, 557.

[3] McCollum, E. V., Newer knowledge of nutrition, New York, 1918, 75.

McCollum this fact has been discussed in terms of the functions of individual amino-acids both as tissue constituents and as possible precursors of hormones and it has been suggested that the protein requirement of maintenance may actually be a requirement for certain amino-acids for transformation into hormones, that the repair processes of maintenance may not involve the disruption and resynthesis of entire protein molecules, or that some of the amino-acids may be used over again in the repair processes. It may also be helpful to think of the protein metabolism not only in terms of building and repair, but also of maintaining the equilibrium which exists between proteins and amino-acids in the cells of the animal tissues. In such a cell there is a constant tendency toward removal of amino-acids by deamination, offset by the inflow of amino-acids derived from food protein and brought to the tissue cell by the blood stream. Concentration of any of the amino-acids into which tissue proteins tend to be hydrolyzed may therefore be expected to help in pushing the reaction, **amino-acids $\rightleftarrows$ protein,** toward the right; in other words, *any* of these amino-acids will function in the *maintenance* of body protein, whereas for the synthesis of new protein as in *growth*, *all* the amino-acids would be needed. Hence it is quite reasonable that proteins of very different efficiency for growth may show much more nearly equal efficiency in the normal maintenance nutrition of adults.

It is well known that as a group the proteins of the cereal grains are less rich in certain amino-acids essential to animal tissue, notably lysine and tryptophane, than are several other of the food proteins; and it is reasonable to expect that such differences in chemical structure among proteins imply corresponding differences in nutritive efficiency. But deficiencies established by experiments with isolated proteins do not necessarily imply corresponding deficiencies in the nutritive value of the natural mixtures of proteins found in our ordinary articles of food. Most experiments designed to compare the nutritive efficiencies of the proteins of different foods have been performed upon laboratory animals during growth, since the nutritive requirements of growth naturally tend to accentuate the differences of food value among proteins which the experiments are designed to discover or demonstrate. Largely for the same reason, however, there is

danger that generalizations from such experiments may lead to exaggerated impressions. On the other hand, we would avoid giving an exaggerated impression in the other direction when we point out that any amino-acid such as results from hydrolysis of body protein may be expected to function in the maintenance metabolism. It is not probable that a molecule of tissue protein is hydrolyzed into a great number of molecules of amino-acids, of for example, fifteen different kinds, in a single step. Autolysis experiments indicate rather that there are successive splittings more or less similar to those which occur in digestive proteolysis, with liberation of amino-acids throughout the process as well as at the end. Any one amino-acid, then, could be expected to check the process only at the point at which that amino-acid would be liberated from the catabolizing protein molecule. If, however, we feed a protein which furnishes considerable amounts of, for example, twelve out of fifteen of the amino-acids in question, then even though the three which are lacking are strictly essential there will still be twelve chances in fifteen of checking the catabolism of the body protein at an early stage, with corresponding efficiency of the incomplete food protein in the protein metabolism of maintenance.

The efficiencies actually found in our typical experiments in which the protein consumed was almost entirely in the form of ordinary wheat bread, or of hard bread made from corn-meal or oatmeal, may be summarized as follows.

Subject R, a man of 80 kilos, established nitrogen equilibrium on a diet of 400 gm. of white wheat bread, 200 gm. of butter, and 300 gm. of apple per day furnishing in all 2,700 calories and 6.0 gm. of nitrogen of which latter 96 per cent was in the form of bread. Thus 37.5 gm. of protein for a man of 80 kilos, corresponding to 33 gm. for a man of average weight (70 kilos), sufficed for the maintenance of normal equilibrium.

Subject O, a woman of 55 kilos, maintained approximate equilibrium (losing less than 0.5 gm. of nitrogen per day) on a diet of corn-meal, butter, sugar, and apple which furnished 2,030 calories and 4.36 gm. of nitrogen per day, all the latter being maize protein except the small amount furnished by the apple. The actual intake of protein was 27 gm. per day. This corresponds to 34 gm. of protein per 70 kilos of body weight per

day. When 100 gm. of milk were added to the diet, making the actual intake of protein 30 gm. (equivalent to 37 gm. per 70 kilos) of which 88 per cent was from corn-meal, 10 per cent from milk, and 2 per cent from apple, the protein supply proved more than sufficient and the subject stored nitrogen. To compare these data with those of the preceding series would somewhat unduly favor the wheat in the comparison since Subject R was considerably older than Subject O and carried a larger proportion of adipose tissue. Under the circumstances, therefore, it seems proper to conclude that the corn protein has shown itself equally efficient with that of wheat in these experiments.

The experiments upon the efficiency of the proteins in oatmeal were carried out with two young women as subjects. The first, Subject O, weight 55 kilos, was the same who had served in the corn-meal experiments just described. The second, Subject P, weighed 67 kilos. Corn-starch was mixed with the oatmeal in such proportions that the cereal part of the diet should furnish practically the same proportions of protein, starch, and calories as in the experiments with corn-meal. The other foods of the experimental diet were substantially as in the corn-meal experiments. Subject O here took the same amount of oat protein which she had taken of corn protein previously. Subject P took amounts of food approximately in proportion to her higher body weight. The main periods of the oatmeal experiment were made parallel to that in which Subject O had taken with the corn-meal diet 100 gm. of milk per day and had stored a small amount of nitrogen. With the oatmeal diet and 100 gm. of milk, Subject O showed nitrogen equilibrium and Subject P a slight storage. In the shorter periods without milk the losses of body nitrogen though small were slightly larger than in the corresponding experiments with corn-meal. It is doubtful if the differences are larger than may be due to the unavoidable fluctuations of nitrogen output in such experiments. The results indicate therefore that the protein of the oatmeal was practically as efficient as the protein of wheat flour or corn-meal.

With all three of the cereal grains tested (wheat, maize, and oats) it was found that a diet, in which about nine-tenths of the protein was derived from the cereal in the form commonly used as human food, the small remainder being furnished by milk or

apple, need contain only 33 to 40 gm. of protein per 70 kilos of body weight, or about 0.5 gm. per kilo, in order to meet the protein requirement of maintenance in adult human nutrition.

The proteins of wheat, corn, and oats appear to be about equally efficient in adult human nutrition and need only be supplemented with small amounts of milk in order to be fully as efficient as the proteins of ordinary mixed diets have been found to be in earlier investigations. Our findings for these cereal proteins are therefore similar to that of Hindhede for wheat bread and show their efficiency to be much higher than was reported by Karl Thomas for either wheat or maize.

Thus from the more recent and more carefully controlled experiments it appears that, even when the protein of the food is almost entirely derived from bread or other grain products, with a diet adequate in energy value a daily intake of about 0.5 gm. of protein per kilo of body weight is sufficient to meet the actual requirements of maintenance in healthy men and women. While if numerous older experiments having a tendency to high results are included the average is somewhat less than $\frac{2}{3}$ gm. of protein per kilo of body weight. A standard allowance of 1 gm. of protein per kilo of body weight per day appears, therefore, to provide a margin of safety of 50 to 100 per cent as far as the requirements of adult maintenance are concerned.

It is plainly desirable in all cases that grain products be supplemented by milk products, and it is clear that in providing for needs of growing children and of pregnant or nursing mothers the proportion of milk in the diet should be more liberal than it need be when only maintenance is concerned; this both because of the superior amino-acid make-up of the milk proteins and to provide amply for the mineral elements and vitamines as well.

EFFECT OF IODIDES ON THE AUTOLYSIS OF LIVER TISSUE.

By P. G. ALBRECHT.

(*From the Laboratory of Physiological Chemistry, College of Medicine, University of Illinois, Chicago.*)

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INTRODUCTION.

The medical literature affords much information concerning the therapeutic value of iodides among which may be mentioned germicidal action, stimulation of the production of opsonins, causation of leucocytosis, increased flow of lymph, and promotion of absorption by increasing the permeability of the vessel walls, but very little can be gathered regarding the chemical changes which the various iodine compounds undergo in the body. Their action on the tissue of the liver, lung, and spleen has been studied by Kepinow (1), but faulty technique has in general yielded inconclusive results as Kaschiwabara (2) demonstrated. Administration of KI was found to accelerate autolysis by Stookey (3) and Kepinow. KI added directly to comminuted liver tissue gave no or little acceleration. The slight acceleration is attributed by Morse (4) to increased acidity from liberation of HI. It is difficult to see how this could occur and no experimental proof is furnished. Considering the question of autolysis from the standpoint of protein cleavage, the literature offers but little information. I have therefore undertaken to examine the effects of NaI on the autolysis of liver proteins.

EXPERIMENTAL.

In order to make the results of the investigation comparable with those of others, the methods of Bradley and Morse (5) were employed with the introduction of a few minor modifications; first, instead of filtering the digest through filter paper it was

TABLE I.

NaI solution, *per cent*		0.03	0.03	0.05	0.1	0.1+	0.25+	0.5+	0.75+	1.0+	1.5+	2.0+
Total nitrogen, *cc.*		13.0	13.0	13.0	11.10	11.90	11.90	11.90	11.90	11.90	11.90	11.90
	days											
Dissolved nitrogen, control, *cc.*	1	3.75	3.60	3.82	3.25	3.50	3.50	3.50	3.50	3.50	3.50	3.50
	2	5.96	6.00	5.60	4.60	5.40	5.40	5.40	5.40	5.40	5.40	5.40
	4	6.66	6.46	6.36	4.70	6.00	6.00	6.00	6.00	6.00	6.00	6.00
	6	7.00	7.00	6.76	5.20	7.16	7.16	7.16	7.16	7.16	7.16	7.16
	8	7.50	7.40	7.00	5.40	7.66	7.66	7.66	7.66	7.66	7.66	7.66
Dissolved nitrogen +NaI solution, *cc.*	1	3.75	3.60	3.82	3.25	3.50	3.50	3.50	3.50	3.50	3.50	3.50
	2	5.60	5.76	5.60	4.86	5.40	6.20	6.00	5.80	5.80	5.50	5.36
	4	6.56	6.36	5.90	5.00	6.10	6.06	6.30	6.10	6.20	6.00	6.16
	6	6.90	6.90	6.50	5.16	7.00	6.90	7.20	7.10	6.60	6.66	7.20
	8	7.36	7.36	6.96	5.56	7.60	7.46	8.00	7.56	7.40	7.36	7.76
Dissolved ammonia nitrogen, control, *cc.*	1	0.80	0.80	0.80	0.80	0.80	0.80	0.80	0.80	0.80	0.80	0.80
	2	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
	4	1.20	1.20	1.20	1.20	1.20	1.20	1.20	1.20	1.20	1.20	1.20
	6	1.30	1.30	1.30	1.40	1.40	1.40	1.40	1.40	1.40	1.40	1.40
	8	1.50	1.40	1.40	1.40	1.40	1.40	1.40	1.40	1.40	1.40	1.40
Dissolved ammonia nitrogen +NaI solution, *cc.*	1	0.80	0.80	0.80	0.80	0.80	0.80	0.80	0.80	0.80	0.80	0.80
	2	1.00	1.00	0.90	1.00	1.00	1.00	1.10	1.20	1.00	1.00	1.00
	4	1.20	1.10	1.00	1.30	1.30	1.20	1.40	1.40	1.40	1.30	1.40
	6	1.30	1.20	1.30	1.30	1.40	1.40	1.40	1.40	1.40	1.40	1.50
	8	1.40	1.40	1.40	1.50	1.50	1.40	1.50	1.40	1.40	1.40	1.50

Dissolved amino nitrogen, control, *mg.*	1	7.20	6.40	5.60	4.60	5.00	5.00	5.00	5.00	5.00	5.00	5.00
	2	9.20	10.40	9.80	10.80	9.80	9.80	9.80	9.80	9.80	9.80	9.80
	4	12.20	13.00	12.20	12.00	11.20	11.20	11.20	11.20	11.20	11.20	11.20
	6	15.00	15.20	16.60	13.60	13.40	13.40	13.40	13.40	13.40	13.40	13.40
	8	15.80	16.20	17.00	15.80	16.60	16.60	16.60	16.60	16.60	16.60	16.60
Dissolved amino nitrogen +NaI solution, *mg.*	1	7.20	6.40	5.60	4.60	5.00	5.00	5.00	5.00	5.00	5.00	5.00
	2	8.60	10.80	10.80	10.60	9.40	10.20	10.00	10.20	10.40	9.80	9.20
	4	11.80	13.00	13.00	12.40	11.20	12.20	12.20	11.20	13.40	12.00	13.60
	6	14.60	15.80	16.20	14.00	15.60	15.40	14.60	14.60	14.20	13.40	13.60
	8	16.00	16.80	17.20	15.20	16.40	15.60	16.60	16.80	15.40	13.80	13.80
Cleavage nitrogen control, *cc.*	2	2.06	3.20	3.10	1.75	2.75	2.75	2.75	2.75	2.75	2.75	2.75
	4	4.20	4.16	4.66	2.35	3.75	3.75	3.75	3.75	3.75	3.75	3.75
	6	5.00	5.00	5.00	2.55	4.51	4.51	4.51	4.51	4.51	4.51	4.51
	8	5.36	5.30	5.36	3.15	5.21	5.21	5.21	5.21	5.21	5.21	5.21
Cleavage nitrogen +NaI solution, *cc.*	2	1.90	3.20	3.20	1.75	2.71	2.65	2.65	2.65	2.75	2.75	2.75
	4	4.20	4.20	4.60	2.35	3.81	3.95	3.81	3.81	3.91	3.85	3.81
	6	4.90	4.90	5.06	2.75	4.41	4.65	4.51	4.45	4.55	4.21	4.71
	8	5.36	5.40	5.40	3.01	5.25	5.35	5.15	5.15	5.21	4.61	5.25
Cleavage ammonia nitrogen, control, *cc.*	2	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
	4	0.40	0.40	0.40	0.50	0.40	0.40	0.40	0.40	0.40	0.40	0.40
	6	0.50	0.40	0.50	0.60	0.60	0.60	0.60	0.60	0.60	0.60	0.60
	8	0.70	0.50	0.50	0.70	0.60	0.60	0.60	0.60	0.60	0.60	0.60

TABLE I.—*Concluded.*

NaI solution, *per cent*.....		0.03	0.03	0.05	0.1	0.1+	0.25+	0.5+	0.75+	1.0+	1.5+	2.0+
Total nitrogen, *cc*.....		13.0	13.0	13.0	11.10	11.90	11.90	11.90	11.90	11.90	11.90	11.90
	days											
Cleavage ammonia nitrogen +NaI solution, *cc.*	2	0.20	0.10	0.20	0.20	0.20	0.30	0.20	0.30	0.20	0.20	0.20
	4	0.40	0.40	0.30	0.40	0.70	0.50	0.60	0.70	0.60	0.60	0.60
	6	0.60	0.50	0.40	0.60	0.70	0.60	0.60	0.70	0.60	0.60	0.70
	8	0.70	0.60	0.60	0.80	0.70	0.70	0.60	0.80	0.60	0.60	0.70
Cleavage amino nitrogen, control, *mg.*	2	1.80	2.00	3.60	2.80	3.80	3.80	3.80	3.80	3.80	3.80	3.80
	4	4.40	5.40	6.60	6.20	9.20	9.20	9.20	9.20	9.20	9.20	9.20
	6	7.80	7.20	10.20	8.80	10.60	10.60	10.60	10.60	10.60	10.60	10.60
	8	8.20	9.40	11.00	11.60	13.60	13.60	13.60	13.60	13.60	13.60	13.60
Cleavage amino nitrogen +NaI solution, *mg.*	2	2.00	2.80	3.00	3.40	4.00	4.20	4.20	4.20	3.60	3.20	4.20
	4	4.80	5.20	6.00	6.60	8.60	8.60	9.20	8.20	10.00	8.40	8.80
	6	8.00	7.80	9.60	8.60	10.80	11.80	9.80	11.00	10.60	10.60	11.20
	8	8.40	9.20	11.40	12.00	12.20	12.80	12.80	13.00	11.80	10.60	11.40

centrifuged until the supernatant liquid was free from visible solid particles, and second, the tannin precipitation method (6) was replaced by the aluminium cream method (7). Ammonia and amino-acid production during the process of liver autolysis in presence of iodides has never been determined.

DISCUSSION.

According to Morse, KI solution of a concentration of 0.2 to 1.0 per cent slightly accelerates autolysis, with inhibition above 1.0 per cent. The results of this investigation confirm his findings only to some extent.

The results in Table I are expressed in cc. of 0.2 N HCl, except that amino nitrogen is expressed in mg. per 1 gm. of fresh liver tissue. The figures in Column 2 indicate the intervals of days: 1 = immediately; 2 = after 2 days; 4 = after 4 days; 6 = after 6 days; 8 = after 8 days.

Dissolved nitrogen (total), dissolved ammonia nitrogen, and dissolved amino nitrogen were determined in the centrifugalized digest.

Cleavage nitrogen (total), cleavage ammonia nitrogen, and cleavage amino nitrogen were determined in the filtrate from the aluminium cream precipitation method.

The results in Table II are obtained by subtracting the control figures from those of the iodides, and the result is multiplied by 100. In concentrations of sodium iodide from 0.1 to 1 per cent an increase is observed especially in the dissolved nitrogen, in the earlier days of incubation. Higher iodide concentration, from 1.5 to 2 per cent, tends also to give higher figures for this nitrogen. However, this proves by no means that NaI is responsible for this increase as far as its action as stimulus for proteolysis is concerned. On the contrary if sodium iodide behaves as other inorganic neutral salts do, according to Preti (8), it would inhibit instead of accelerate proteolysis, when employed in these concentrations. There is further a possibility that the ionized sodium iodide is responsible for the formation of soluble ion-protein compound which behaves in many respects as soluble protein.

A slight variation in dissolved ammonia nitrogen and cleavage ammonia nitrogen is noticed, which I am not in a position to explain.

TABLE II.

NaI solution, *per cent.*		0.03	0.03	0.05	0.1	0.1	0.25	0.5	0.75	1.0	1.5	2.0
	days											
Dissolved nitrogen (N), *cc.*	2	−36	−24	0	26	6	80	60	40	40	10	−4
	4	−10	−10	−46	30	10	6	30	10	20	0	16
	6	−10	−10	−26	−4	−16	−26	4	−6	−56	−50	4
	8	−14	−4	−4	16	−6	−20	34	−10	−26	−30	10
Dissolved ammonia nitrogen (NH_3−N), *cc.*	2	0	0	−10	0	0	0	10	-20	0	0	0
	4	0	−10	−20	10	10	0	20	20	20	10	20
	6	0	−10	0	10	0	0	0	0	0	0	10
	8	−10	0	0	10	10	0	10	0	0	0	10
Dissolved amino nitrogen (NH_2−N), *mg.*	2	−60	40	100	−20	−40	40	20	40	60	0	−60
	4	−40	0	80	40	0	100	100	0	220	80	240
	6	−40	60	−40	40	220	200	120	120	80	0	20
	8	20	60	20	−60	−20	−100	0	20	−120	−280	−280
Cleavage nitrogen (N), *cc.*	2	−16	0	10	0	−4	−10	−10	−10	0	0	0
	4	0	4	−6	0	6	20	6	6	16	10	6
	6	−10	−10	6	20	−10	14	0	−6	4	−30	20
	8	0	10	4	−14	4	14	6	6	0	−60	4
Cleavage ammonia nitrogen (NH_3−N), *cc.*	2	0	−10	0	0	0	10	0	10	0	0	0
	4	0	0	−10	−10	30	10	20	30	20	20	20
	6	10	10	−10	0	10	0	0	10	0	0	10
	8	0	10	10	10	10	10	0	20	0	0	10
Cleavage amino nitrogen (NH_2−N), *mg.*	2	20	80	−60	60	20	40	40	40	−20	−60	40
	4	40	−20	−60	40	−60	−60	0	−100	80	−80	−40
	6	20	60	−60	−20	20	120	−80	40	0	0	60
	8	20	−20	40	40	−140	−80	−80	−60	−180	−300	−220

The results of dissolved amino nitrogen are high compared with the cleavage amino nitrogen. This stands to reason, taking into consideration the methods employed. Aluminium cream will precipitate colloidal material, which will not be removed by the centrifugalization method, hence the figures of the latter are higher.

CONCLUSION.

It is evident from the results of this investigation that sodium iodide in concentrations of 0.03 to 2 per cent added to a suspension of liver pulp has no effect on the autolysis of this tissue.

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I. A CHEMICAL STUDY OF THE BLOOD OF SEVERAL INVERTEBRATE ANIMALS.*

By ROLLIN G. MYERS.

(From the Department of Chemistry and Hopkins Marine Station of Stanford University, Palo Alto.)

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A study of the literature reveals the existence of comparatively few communications related to this particular field. The earliest work appears to be that of Macallum (1), dealing with the inorganic constituents of the blood of vertebrates and invertebrates. Other investigations somewhat closely related were made by Lipschütz (2), on the metabolism of fishes during starvation; Botazzi (3), on the peritoneal fluid and blood of sea animals; Jona (4), on freezing point values of the blood and body fluids of certain mammals, fish, and crustacea; Okuda (5), dealing with the quantitative determination of creatinine and creatine in some fishes, mollusks, and crustacea; and Fandard and Ranc (6), on sugar in the blood of the sea turtle.

The results embodied in these communications are greatly limited, however. The earlier workers were handicapped by the lack of accurate methods, and hence could not extend their studies to any great length. In later years the only work which has a comparative value when studied with that of the present paper is that of Denis (7), and of Wilson and Adolph (8), dealing with the determination of certain nitrogenous substances in the blood of fresh and salt water fishes; *e.g.*, shark, ray, mackerel, carp, and a few others.

In view of the general lack of information concerning the composition of the blood of invertebrate animals, it is evident that any substantial addition to our knowledge in this field

* This paper is a part of a thesis presented to the Department of Chemistry of Stanford University in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

would be of distinct interest. The immediate object of this investigation was the collection of chemical data along this line, with the added purpose of making a comparative study of the data obtained with relation to that from the more highly evolved animals. It is hoped that this study may bring to light a certain number of general relationships, which will enlarge our ideas of general metabolism, and, more remotely perhaps, assist the zoologist in tracing out some of the less clear biogenetic relationships.

The following invertebrate forms were studied.

Cœlenterates: The jellyfish, *Phacellophora camtschatica.*

Echinoderms: Two species of starfishes, *Pisaster ochraceus* and *Picnopodia helianthoides.* One species of sea urchin, *Strongylocentrotus francescames.*

Mollusks: Two species of clams, *Schizotherus nuttalli* and *Saxidomus nuttalli.* Two species of abalone, *Haliotis rufescens,* and the supposedly primitive molluscan form *Cryptochiton stelleri.*

Crustaceans: Two species of crabs, *Cancer productus* and *Cancer antennarius.* In this case the blood of the two species was mixed.

All the samples of blood were collected from the living animal, (*a*) by exposing the deeper surfaces, (*b*) by severing wholly or in part a blood vessel, and (*c*) by maceration of the tissues.

Where it was possible a specific gravity determination was made, and this was followed by a quantitative and qualitative chemical examination. The quantitative determinations made were total nitrogen, non-protein nitrogen, urea and ammonia nitrogen, amino-acid nitrogen, preformed and total creatinine, uric acid, sugar, cholesterol, chlorides as sodium chloride, calcium as calcium oxide, total solids, and ash. The qualitative tests included the reaction to litmus, the biuret, Millon's, Hopkins-Cole, xanthoproteic tests, and that for loosely combined sulfur.

In general the chemical examination was made directly after the blood was collected. Oxalate or citrate was added only in those cases in which experience had shown that coagulation would occur before the analysis could be undertaken. The samples were kept in tightly stoppered containers which were placed on ice. Toluene was added when conditions made this step advisable. Quantitative determinations were usually run in dupli-

cate, sometimes in triplicate. Analyses were repeated in all cases where there was the least doubt of the accuracy of the results. Relatively complete analyses were made, when the quantity of blood in a given sample permitted this to be done. In several cases repeated analyses of the blood from the same species of animal, collected at different times, were conducted. Whole blood was used in every case, and was composite, from two or nineteen or twenty forms of the same species being represented in the samples analyzed.

The methods followed in the collection of the samples were different for the various species studied. While the jellyfish, *Phacellophora*, possesses no celomic fluid or blood, it was included on account of its zoological position. Consisting largely of amorphous jelly-like mesoglea, quite firm to the touch, it was readily reduced to a fairly homogeneous liquid by gentle maceration through cheese-cloth.

In the case of the starfish, as much as possible of the extraneous sea water was removed, and then several of the rays were clipped off a few centimeters from their distal ends. The exuding liquid was allowed to drain into vessels arranged for the purpose. In the later samples the liquid was filtered through a loose tuft of absorbent cotton.

The procedure was slightly different for the sea urchin. In this case several incisions were made in the membrane surrounding the oral cavity. The animal was drained in the manner previously described, and the liquid filtered as before.

On account of the rather definite circulatory system of the mollusks, the procedure used in the preliminary stages of the work was limited to the selection of some of the larger blood vessels. Later on, however, a quicker and easier method was devised. Rather deep cavities were cut in the foot, for example, of the abalone, *Haliotis rufescens*, or in the gilt-cleft of the *Cryptochiton stelleri*. The blood filled these cavities rapidly and was removed by means of a small pipette with a curved end connected with a suction flask. Or in the case of the abalone, a part of the shell was broken and one of the branchial vessels exposed. This was partially severed, a fairly large hypodermic needle inserted, held securely in place with a small pair of forceps, and the blood carried over into a flask by suction. For

some unknown reason this method did not always yield an abundant supply of blood. A similar method was used for clams. The shell was broken and the position of the heart determined. The pericardial membrane was then severed as well as the superior vena cava and the blood running into the cavity removed in the manner previously described. This, however, is a tedious process. In order to facilitate matters the exposed body of the clam was superficially cut in several places and then gently macerated. The liquid collected was filtered through loose cotton. Total nitrogen determinations made showed a value of 39.98 mg. per 100 cc. of blood obtained in this manner, as compared with 39.92 mg. in blood collected from the pericardial cavity. Thus the difference is slight and the maceration method is to be preferred for simplicity as well as quickness.

The methods used for collecting crab blood were not essentially different from those previously described. Coagulation of the blood of these animals proceeds very rapidly after it is drawn—fibrin frequently separating out before sufficient oxalate is dissolved to prevent it.

Methods of Analysis.

Methods for blood analysis have thus far had a rather narrow application. The protein nitrogen for vertebrate blood has varied in round numbers from 2,800 to 3,700 mg. per 100 cc. of blood. The limits of the mineral constituents perhaps could be truthfully set at 400 to 600 mg. per 100 cc. These values are in decided contrast to those obtained in this investigation from the blood of invertebrate animals. Here the limits for the protein nitrogen vary from 1.5 mg. in the twenty ray starfish to 358 mg. in the crab, and for the mineral constituents from approximately 2,500 to 3,000 mg. per 100 cc. in the various species studied. In fact, comparatively speaking, these numbers are just the reverse in magnitude of those for the common vertebrates. In view of these striking differences, the presumption appears to favor the conclusion that occasional changes in the ordinary analytical procedures would be necessary. Experience in general has justified this view.

For preliminary removal of proteins the 50 per cent solution of trichloroacetic acid, used by Greenwald (9) and others, was by far the most satisfactory precipitant examined.[1] In some instances one precipitation was all that was necessary, the filtrate remaining free from any insoluble matter after considerable concentration. In many cases, however, it was necessary to digest the mixture in boiling water for 15 or 20 minutes to obtain the best effects. Enough of the acid was used to produce a concentration of from 6 to 8 per cent.

The freshly prepared 25 per cent solution of *m*-phosphoric acid recommended by Folin and Denis was not so satisfactory. The filtrates were in general cloudy, and even digestion in boiling water, a procedure which might be questioned, failed to accomplish the end desired. Concentration seemed to be the only recourse. There is, of course, the possibility always present of a union between the acid or some of its derivatives and the protein to form soluble products. This effect is very likely to take place when *m*-phosphoric acid is used, according to Folin and Denis, particularly where the acid is not fresh. Heating would of course favor this condition. Whether trichloroacetic acid would react similarly does not seem to be known, but experience seems to be against such a possibility. In any case any solution of the protein would vitiate the whole procedure.

Picrate-picric acid solution proved a most excellent precipitant, but its special character limited its use.

Alcohol was also used, but evaporation always yielded appreciable quantities of protein.

0.01 N acetic acid as a general precipitant was to be criticized in much the same way as *m*-phosphoric acid.

Kaolin as an absorbent of protein was satisfactory.

For total nitrogen, the Kjeldahl-Gunning method was followed, a small quantity of copper sulfate serving as catalyzer.

Non-protein nitrogen was determined by the method of Folin and Denis (10) and also by applying the Kjeldahl method to the protein-free filtrate. On account of the difficulty attendant on the absorption of

[1] The recently described protein precipitant for use in blood analysis, tungstic acid, recommended by Folin and Wu (Folin, O., and Wu, H., *J. Biol. Chem.*, 1919, xxxviii, 81), was not used, since the investigation was nearly completed when the method came to the notice of the author.

color by the silicious insoluble matter, most of the determinations were made by the latter method. In most cases a sufficient supply of blood permitted this choice.

The determination of urea nitrogen was made by the methods of Folin and Denis (11) and Van Slyke and Cullen (12). Latterly the first of these methods was preferred, but with certain modifications. Instead of an emulsion of soy bean, a urease preparation was used either in the form of a powder or of a phosphate solution, according to Van Slyke and Cullen. The blood with the urease was digested at 45–50°C. for 30 minutes or longer, the remaining procedure being similar to that of Folin and Denis, except that 10 cc. of the filtrate were aerated into a known volume of a 0.2 N sulfuric acid solution to which was added ammonium sulfate equivalent to 0.05 mg. of nitrogen per cc., explanation of which will be made later. To expel the ammonia, 15 cc. of saturated potassium carbonate solution were used. The absorbent was then Nesslerized in the usual manner, a 100 cc. volumetric flask being used. The color comparisons were made by a Duboscq colorimeter with the standard set at 20 mm. Control determinations were made by subjecting urea solutions of known concentration to the action of urease, and completing the determination in the same manner as the unknown.

The determination of ammonia nitrogen was made according to the method of Folin and Denis (13) though with considerable modification influenced to some extent by the results of Barnett (14). 10 cc. of the blood were mixed with 15 cc. of saturated potassium carbonate solution and the mixture was aerated into a definite volume of 0.2 N sulfuric acid solution containing 0.05 mg. of ammonium sulfate per cc. The absorbent solution was then Nesslerized according to the method of Folin and Denis, and a colorimetric determination made with the standard set at 20 mm. Since a definite quantity of ammonia nitrogen was used to start with in the absorbent, this was subtracted from the total. The remainder represented the ammonia nitrogen in the blood. As will be noted a similar procedure was used in the determination of urea. The advantages of aerating into a standard solution of ammonium sulfate made acid with sulfuric acid are easily seen in this determination. The accessory apparatus of polarimeter tubes and condenser is eliminated. The depth of shade of the Nessler solution can be varied to suit the eye. Finally Nesslerizations can be made in 100 cc. volumetric flasks and after a little practice the standard can be so adjusted to the unknown that the reading will vary not more than 4 or 5 mm. from 20. The same remarks may well apply for the determination of urea nitrogen. The gain in simplicity is considerable.

In general the author does not look on aeration procedures with any great degree of favor. In spite of protective tubes and capacious aeration cylinders, it was necessary occasionally to make repeated determinations. The distillation procedure is perhaps more preferable.

The observation made by Barnett (14) that the quantity of ammonia nitrogen increases in the blood on standing was followed in its practical

significance by conducting the determination as soon as possible after the blood was drawn. That there is a decided increase in the quantity of ammonia on standing is in general agreement with the author's findings. This, it might be mentioned, is particularly true for whale blood.

Amino-acid nitrogen was determined by means of the Van Slyke (15) micro apparatus. In the initial treatment of the sample, the procedure of Bock (16) was followed. Several determinations were made, but only two are given in the table. In sea urchin, abalone, and crab blood values varying from 30 to 70 mg. per 100 cc. were obtained. The determinations were not always made directly after the blood was drawn. The post-mortem formation of amino compounds, or the action of certain substances in the blood on the nitrite used, might account for these high values. In any case there appear to be certain disturbing conditions.

Preformed and total creatinine were determined according to the method of Denis (17). In some cases 10 cc. of the standard were added to the unknown, and the determination was made. The picric acid used was tested according to the method of Folin and Doisy (18) and was found to satisfy the requirements. The principal difficulty experienced was the failure of color to develop after the prescribed amount of 10 per cent sodium hydroxide was added. Precipitates usually formed after the base was added. In order to overcome the first of these difficulties a cautious addition in excess of from 0.5 to 1 cc. of the base was made. Within narrow limits a variation in the base used had no influence on the reading when the experiment was conducted on standard creatinine solutions of the same concentrations. Precipitates were eliminated by centrifugalization. These difficulties appeared to be accentuated in determinations of total creatinine.

Uric acid was determined by the method of Folin and Denis (19). For amounts of uric acid which are found in human blood or of an approximate magnitude, no trouble was experienced. When only traces of the acid exist, and these in the presence of about 3 per cent sodium chloride, difficulties were encountered which were not entirely overcome. In order to render measurable the traces which appeared to be unmistakably present, from 30 to 50 cc. of the blood were used. The crystallizing out of considerable quantities of sodium chloride on evaporation appeared to vitiate the determination. Definite quantities of uric acid were then added to 3 per cent sodium chloride solution, but it seemed to be impossible to recover anything like the quantity of acid added. The large excess of sodium chloride may hinder perhaps the formation of silver urate by its mass action effect. Indications are that the method must be considerably modified to determine small quantities of uric acid under the conditions described.

Sugar was determined by the method of Lewis and Benedict (20). The picramic acid for the standard was prepared[2] according to the later method

[2] The work of preparation was kindly performed for the author by Dr. E. Oertly of Stanford University.

of Egerer (21). The only difficulty met with was in the application of the method to certain samples of blood such as that of the starfish, where the protein was very low. In this case the precipitate formed was difficult to remove by filtration. The precipitate appeared in the form of a suspension which did not readily settle.

For cholesterol, the method of Myers and Wardell (22) was used. Two modifications were introduced after several experiments. Instead of the standard "napthol green B," a standard solution of Kahlbaum's cholesterol 1.0 mg. per 100 cc. in chloroform was used. The original procedure of taking an aliquot part of the extract was changed and the whole volume was concentrated to 5 cc. This modification was made necessary on account of the small quantities of cholesterol present, even when 3 to 5 cc. of the original blood were used.

It was found that the unknown as well as the standard cholesterol solutions possessed a decided bluish tint after performing the Liebermann-Burchard reaction. Hence good comparisons could not be made with the dye used as a standard. The blue shade observed is in decided disagreement with the observations made by Luden (23) in connection with the same reaction.

A final difficulty, not fully overcome, was the formation of a reddish shade in the extracts. This increased after adding the sulfuric acid and acetic anhydride. The shade seemed to develop to the greatest extent in clam blood. In the light of Luden's work the conclusion may be drawn that this color is due to icteric substances in the blood. At any rate, the color comparisons were made most difficult on account of this condition.

Chlorides as sodium chloride were determined according to the method of Rappleye (24). The method is simple and the end-point sharp for the chlorides in most invertebrate blood. When the sodium chloride is less than 0.6 per cent, the end-point becomes somewhat uncertain. Only 1 cc. of blood was used for the determinations, the amount of the silver nitrate solution varying from 15 to 20 cc. These quantities are slightly different from those prescribed by the method. In the author's view the method is considerably more simple than that of Van Slyke and McLean, and equally as accurate.

For total solids, ash, and calcium as the oxide, 5 to 10 cc. of the blood were evaporated to dryness in a weighed porcelain crucible over a water bath, and the residue was dried to constant weight at 110–115°C. This represented the total solids. The residue was then ignited, the temperature being kept as low as possible to avoid volatilization of the alkalies—a condition not always easy to accomplish in removing the last trace of the carbon—and then weighed. This represented the ash. The latter was then extracted with 15 to 20 cc. of concentrated hydrochloric acid, and the calcium as the oxide determined in general according to the method of McCrudden (25).

Specific gravity determinations were made at 20°C. by a pycnometer bottle.

The values in Table I exhibit several differences when compared with similar determinations made on the more highly developed animals. The exceptionally high content of inorganic salts, 2,900 to 3,200 mg., and the low protein nitrogen content, 0.7 to 340 mg. per 100 cc. of the blood, may be taken in illustration.

The influence of high saline content on osmotic pressure is obvious. The relatively small amount of urea and other organic crystalloids in the samples of blood examined clearly proves that the osmotic pressure is for the most part caused by the inorganic constituents. In any case the osmotic pressure is nearly the same as sea water ($\Delta = 1.81$–$2.8°$) (26). In the more highly developed elasmobranch fishes this pressure is undoubtedly produced to a notable degree by the very large quantities of urea present in the blood, a constituent present in only small quantities in invertebrate blood. Neither the invertebrates examined nor the elasmobranch fishes appear to be independent of the surrounding medium in this regard. Neither set of forms has any means which enable them to influence their own osmotic pressure. This is in direct contrast to the marine and fresh water teleost fishes where probably a reduced saline as well as a urea content effects a material lowering of the osmotic pressure of $\Delta = 0.7°$. As pointed out by Wilson and Adolph (27), these species are partially independent of changes in the surrounding medium. Undoubtedly the gills of these fishes have been modified in some way which enables them to adapt themselves to conditions of this kind.

That the low protein content of the blood of the invertebrate animals has a decided influence on its viscosity can hardly be doubted. Compared with the viscosity of the more highly developed marine forms and the mammals it should be considerably lower. In mollusk and crustacean blood the increased protein content would be parallel to an increased viscosity, and this in turn is associated with an improved cardiac and circulatory apparatus.

The buffer effects of the protein in mammalian blood in maintaining the required hydrogen ion concentration probably prevail in the blood of invertebrates, this value approximately being from 6.4 to 6.7 pH if the alkaline reaction to litmus is considered (Table II).

TABLE I.

*Quantitative Data per 100 Cc. of Blood.**

Animal.	Specific gravity.	Solids.	Ash.	Ca as CaO	Cl as NaCl.	Total N.	Non-protein N.	Urea + NH_3 N.	Urea.	NH_3 N.	NH_2 amino N.	Creatinine. Preformed.	Creatinine. Total.	Creatine.	Uric acid.	Sugar.	Cholesterol.	Remarks.
		mg.	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	
Jellyfish, *Phacellophora camtschatica.*						44	12	5.0				0.01	0.30	0.29		109		Obtained from Monterey Bay, Cal.
5 ray starfish, *Pisaster ochraceus.*				53	3,225	5	4.4	1.0		0		0.01	0.13	0.12			0.9	Obtained from Monterey Bay, Cal.
20 ray starfish, *Picnopodia helianthoides.*				56	3,225	6	4.0	2.0								29		Obtained from Monterey Bay, Cal.
						1.5	0.8	0.5									1.0	
Sea urchin, *Strongglyocentrotus francescames.*	1.024	3,485	2,992	63	3,140	12	8.6	1.0	0.92	0.08		0.11	0.27	0.16	Trace.	61	4.0	Obtained from Monterey Bay, Cal.
Clam, *Saxidomus nuttalli.*						44	11.0					0.28				50		
		4,330	2,800	307		59	13.0	10.0	9.05	0.95		0.31	0.74	0.43		77		Obtained from Moss Landing, Cal.
							10.0	3.3										
Schizotherus nuttalli.	1.029	4,208	3,290	193	3,190	40	14.0	3.6	2.94	0.66	8.0	0.38	0.80	0.42	Trace.	74	2.0	Obtained from Moss Landing, Cal.

Cryptochiton stelleri.	1	1.030					197		3.0	2.00	0.40						41		Single specimen, 120 cc. of blood, 21×11×5 cm.
	2	1.029									0.80								Single specimen, 137 cc. of blood, 19×10×5 cm.
	3	1.032																	Single specimen, 67 cc. of blood, 18×10×4 cm.
1, 2, 3 composite.							231	25.0	4.0	3.5	0.50	13.0	0.27			0	49		
					66	3,094	177	6.5	1.3	1.3	0.00					0	45	3.0	Obtained from Monterey Bay, Cal.
Abalone, *Haliotis rufescens* (red).							100		4.8	4.8	0.00		0.65				38		Obtained from Monterey Bay, Cal.
							200	21.0	6.0	5.60	0.40						37		
Abalone, *Haliotis rufescens* (black).			5,154	2,376	74	3,130	231	23.3	2.4	2.27	0.13		1.25	2.08	0.83		91	3.6	Obtained from Monterey Bay, Cal.
		1.029	4,730		87	3,160	168	15.0	1.4	1.11	0.29		0.31	0.78	0.47	Trace.	61	3.0	
Crab (two species), *Cancer productus.* *Cancer antennarius.*			4,685		73	2,895	358	18.0	6.2	5.15	1.05		0.20	0.78	0.58	4.7	90	5.7	Some clotting took place. Obtained from Moss Landing, Cal.

* A sample of sea water (a mixture of 15 samples representing consecutive days) contained 1,920.5 mg. of Cl (*i.e.* 3,165 mg. of NaCl) and 40 mg. of CaO per 100 cc. Monterey Bay, Cal., near the Hopkins Marine Station. Period April 26, 1919, to May 13, 1919.

TABLE II.

Qualitative Data on Samples of Whole Blood.

Animal.	Color of blood.	Reaction.					
		Litmus.	Xanthoproteic.	Millon.	Hopkins-Cole.	Lead.	Biuret.
Jellyfish, *Phacellophora camtschatica*	Colorless, macerate.	Slightly alkaline.	Medium.	Medium.	Quite strong.	Weak.	Medium.
Cryptochiton stelleri	Dark amber.	"					
Sea urchin, *Stronglyocentrotus francescames*	Orange.	"	Medium.	Medium.	Weak.	Weak.	Medium.
Abalone, *Haliotis rufescens*	Light indigo blue.	"	Quite strong.	Quite strong.	Strong.	Very strong.	Pinkish.
Clam, *Schizotherus nutalli.*	Bluish tint.	"	Medium.	Medium.	Weak.	Quite strong.	Medium.
Crab, *Cancer productus* and *antennarius*	Light greenish blue.	"	Strong.	Quite strong.	Very strong.	Strong.	Pinkish.

The high values for calcium oxide, from 53 to 307 mg. per 100 cc. of blood are in accord with what one might expect in view of the considerable demand on the part of the invertebrate animals for the calcareous matter needed for the shell. The largest amount, 307 mg. per 100 cc. of blood, occurs in the *Saxidomus* clam. The shell of this species is much heavier than that of the associated *Schizotherus* clam. But if this criterion is taken to account for the difference in the amount of the calcium salts observed, it is somewhat difficult to account for the lower values 74 to 87 mg. per 100 cc. in abalone blood. A qualitative observation favors the view, however, that the proportion of the body of the clam to its shell is considerably less than in the case of the abalone.

Compared to several mammals, *e.g.* human blood 9.5 to 11 mg. per 100 cc., the calcium salts found in invertebrate blood may be several times as great.

The proportion of urea nitrogen to non-protein nitrogen appears to vary within rather wide limits. This is true for the echinoderms, 22 to 62 per cent of the non-protein nitrogen being represented as urea nitrogen in the blood of starfishes and 11 per cent in the one sample of sea urchin blood examined. In the mollusks and the one sample of crustacean blood the amounts vary from 7 to 70 per cent, though the mean value would fall more nearly between 10 and 20 per cent. The tendency then seems to be toward conditions similar to those which prevail in the blood of the teleost and ganoid fishes. The additional fact that the urea nitrogen is 40 per cent of the non-protein nitrogen in the flesh of the jellyfish examined has further interest.

The partition of the urea nitrogen between the form elements and the plasma was not studied. The form elements, however, comprise only a small proportion of the total nitrogen of the blood, it is believed. This appeared to be true particularly in the case of *Schizotherus* clam blood. The indications are that the urea is found principally in the plasma.

The ammonia, preformed creatinine, and total creatinine content of the blood of the echinoderms, mollusks, and crustaceans examined was in general considerably lower than the same constituents observed in the elasmobranch, teleost, and ganoid fishes (28). The amount of ammonia seemed to approximate more

closely that found in mammalian blood, though the creatinine and creatine are still lower even in this case. In addition to this the amount of creatine found in invertebrate blood was only a little greater frequently than the preformed creatinine. In starfish blood the creatine appears to be greatly in excess of the preformed creatinine, however.

The occurrence, at most only in minute traces, of uric acid in the blood of most of these animals agrees with the findings of Denis, in respect to the blood of the elasmobranch fishes. In the blood of the crab the amount of uric acid is relatively high, 4.7 mg. per 100 cc., and considerably in excess of that found in human blood; *e.g.*, 0.7 to 3.7 mg. per 100 cc. In this regard the metabolism of the crab appears to be similar to that of the birds and reptiles.

The amino-acid nitrogen probably makes up a large part of the remainder of the non-protein nitrogen, though only two determinations can be given to support this statement.

Sugar was found in all the samples of invertebrate blood examined, and varies from 29 to 90 mg. per 100 cc. of the blood. These values are similar in magnitude to those of human blood, *e.g. 20* to *150*, and perhaps other mammals. The only other determinations of sugar made on invertebrates were those of Fandard and Ranc (29) in the blood of a fasting turtle. Their values range from 82 to 95 mg. per 100 cc. of the blood, which are in agreement with those of the author for abalone and crab blood. The extreme variation does not appear to be wide in any of the animals examined. In respect to the invertebrate blood, it is interesting to speculate on the manner in which these animals maintain the amounts of sugar observed, in the presence of so much sodium chloride. At least in man the injection of 1 per cent salt solution intravenously becomes a glycuretic in causing a decided increase of the blood sugar in the urine. The relatively large amounts of calcium salts in the blood of invertebrates may lessen the permeability of the excretory membranes as they do apparently in man (30).

The quantity of cholesterol found was small in all the bloods examined, the variation extending from about 1 to 6 mg. per 100 cc. of blood. Compared with the values given for human blood (30 to 60 mg. per 100 cc.) they appear to be very low.

There seems to be considerable scarcity of data for cholesterol in the blood of mammals as well as of fishes. Hence any comparison must be extremely limited.

Finally it is of extreme interest to note the occurrence of several of the combined amino-acids, *e.g.* tyrosine, tryptophane, and cystine, in the tissue and the blood of these marine forms (Table II). If the qualitative tests are significant, the relatively large amounts of combined cystine and tryptophane in abalone and crab blood are most interesting.

SUMMARY.

The following determinations were made on the blood of several invertebrate animals: Specific gravity, total solids, calcium as calcium oxide, chlorine as sodium chloride, total nitrogen, non-protein, urea, ammonia, and amino-acid nitrogen, preformed and total creatinine, uric acid, sugar, and cholesterol. These values are found in Table I.

The osmotic pressure of the blood of these animals is about the same as sea water, and in this respect they agree with the elasmobranch fishes; *e.g.*, shark and ray. The major part of the osmotic pressure is due to the high saline content and not to the urea as in the elasmobranch fishes.

The very possible influence of low protein content in the blood on the lowered viscosity and the probable increase of this factor in the blood of the Mollusca and Crustacea is accompanied by a more highly developed circulatory system.

The urea content is relatively low, though the mean value is higher than that observed in some of the marine and fresh water fishes.

The ammonia, preformed creatinine, and creatine were generally lower than in the blood of the vertebrate fishes and other mammals.

The amino-acid nitrogen probably makes up the larger part of the non-protein nitrogen.

Sugar was found in the blood of every invertebrate examined and in relatively large quantities. In comparison with the sugar in the blood of other forms, the differences were not great.

Cholesterol was found in very small quantities. In comparison with the blood of man, the amounts were low.

The combined amino-acids, tyrosine, cystine, and tryptophane, the two latter in considerable quantities, were shown to be present in most of the blood(s), and in one case the tissue, of the invertebrate animals examined.

In conclusion the author wishes to express his great indebtedness to Professor R. E. Swain, whose many suggestions and practical assistance had a most happy influence on the success of this investigation as well as that connected with whale blood reported in the following paper.

In connection with the collection of the blood samples, the author wishes to acknowledge also the invaluable assistance of Professor Walter K. Fisher of the Hopkins Marine Station of Stanford University, Monterey, California.

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II. A CHEMICAL STUDY OF WHALE BLOOD.*

By ROLLIN G. MYERS.

(*From the Department of Chemistry and Hopkins Marine Station of Stanford University, Palo Alto.*)

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A study of the literature revealed an almost complete lack of any work done in connection with the chemistry of whale blood. There seems, indeed, to be a general lack of information in respect to the zoological side as well. This paper then becomes an entirely new addition to the chemical knowledge of the whale, in particular to that of its blood. While not extensive, as far as repeated analyses are concerned, the work at least is a beginning for more extended examinations.

Two species only are included; the humpback whale *Megaptera versabilis* Cope, and the sperm-whale *Physeter macrocephalus* Linneus.

It was impossible to obtain the blood directly after the animal was killed. 3 or 4 hours, sometimes more, frequently elapsed before the sample was obtained. The blood was obtained from the freshly exposed and deeper muscular regions of the animal. The blood gushed from these regions in large quantities and no difficulty was experienced in getting it. It was quite warm and clotting ensued only some time after it was drawn. The ordinary methods failed to recover any fibrin from the blood of the humpback whale. The amount obtained from sperm-whale blood was relatively small compared to the blood used. Oxalate was added, except in samples where total solid determinations were made. The first humpback whale sample was taken from the thoracic cavity. The blood is probably not very pure, but it was decided to include it in the tabulation.

* This paper is part of a thesis presented to the Department of Chemistry of Stanford University in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

TABLE I.

Analyses per 100 Cc. of Whale Blood.

Animal.	Date.	Specific gravity.	Total solids.	Ca as CaO.	Cl as NaCl.	Total N.	Non-protein N.	Urea + NH_3 N.	Urea.	NH_3 N.	NH_3-acid N.	Creatinine.		Creatine.	Uric acid.	Sugar.	Cholesterol.	Remarks.
												Preformed.	Total.					
	1919		*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	
Humpback whale, *Megaptera versabilis* Cope. 1*	Mar. 24					1,177	325	160	156	4.0		2.2				400		Killed in Monterey Bay, Cal.
2†	" 11	1.038		15.8	580	2,237	221	137	122.5	14.5	59	17.6	39.2	21.6	5.1	266	42.6	Killed in Monterey, Bay, Cal.
Sperm-whale, *Physeter macrocephalus* Linneus.‡	May 1	1.061	22,025	13.7	509	3,518	116	74	71.6	2.4	31	5.3	14.2	8.9	2.1	118	65	Killed in the Pacific Ocean about 10 miles off Monterey Bay, Cal. The amount of protein by the Thierfelder method was 20,775 mg., the protein ash 195 mg., and soaps and lipins including cholesterol approximately 401 mg. per 100 cc.

* The blood was probably impure, since it was selected from drainings into the thoracic cavity.

† This animal was about 40 feet long and had an estimated weight of 35 tons. This species belongs to the whale bone whales. A larger species is the so called "sulfur bottom" or baline whale. The latter grow to the length of 95 feet. The humpback feeds on large quantities of sardines and anchovies. Their stomachs have been known to contain from 2 to 3 tons of these fish.

‡ The specimen from which the blood was obtained had a length of 65 feet 9 inches. The lower jaw was about 8 to 9 feet in length, and had 48 teeth 2 to 3 inches in diameter, and was narrow and tapering. The head was about one-third the length of the whole body. The weight of the animal was variously estimated at from 80 to 90 tons. Blubber was 6 to 8 inches in thickness. Of the spermaceti recovered, there were 90 barrels from the whole animal, 32 barrels coming from the head alone. The exposed intestines had a dark, though bright fluorescent green color. The stomach contained a shark 10 feet 2 inches long, the pigmented layer of whose skin only having been attacked by the gastric juices, the skin of a partially digested seal, and 8 feet of fishing line with six hooks. While the flesh of the humpback whale is used for food, the flesh of this species is not. The sperm-whale appears to be entirely carnivorous in habits, squid and devil fish being favored articles of diet. When wounded it will attack the pursuing boat, according to reports. The author was favored with most of the above data by Mr. Chester I. Dennis, an official of the California Sea Products Company, Moss Landing, Cal.

The methods of analysis employed were the same as those used in the examination of invertebrate blood (see page 122). Little if any difficulty was experienced in the application of these methods.

In contrast to invertebrate blood, *m*-phosphoric acid solution gave clear filtrates. These, however, precipitated small quantities of protein on evaporation.

In the cholesterol determinations the reddish shade noted in the previous paper was also present in the extracts of whale blood.

A study of Table I reveals noteworthy differences in the composition of humpback and sperm-whale blood. Not only are these differences exhibited in the protein content, and in the nitrogen partition, but in some of the other constituents as w ll. Our general ignorance of the habits and life of these animals renders somewhat abortive all attempts to account satisfactorily for these differences. Both animals appear to be carnivoral feeders, though the sperm-whale, in contrast with the humpback, devours larger animals. Many other conditions would have to be considered, however, and the whole question will remain obscure until our knowledge becomes more definite and extended.

In respect to the humpback whale the large quantity of urea in the non-protein fraction would have a considerable influence on the osmotic pressure of the blood. This would be considerably lower than that of sea water, of invertebrate and elasmobranch fish blood, though higher than that of the marine and fresh water teleost fishes. The value $\Delta = 0.8 - 0.9°$, would perhaps be an approximation. The blood of the sperm-whale is considerably lower than this, the range of this value being similar in magnitude to that of the teleost fishes ($\Delta = 0.7°$). The osmotic pressure of the blood of both species however is higher than that of the blood of several of the land mammals; *e.g.*, $\Delta = 0.6°$. Evidently both species are relatively independent of changes in the medium, which would give them a wide foraging capacity. It is somewhat interesting in this connection to specu'ate on the ability of the whale to adapt itself to a fresh water medium.

The striking contrast in the amounts of protein in the blood of the humpback (13,981 mg.) and the sperm-whale (20,775 mg.

per 100 cc.) suggests interesting possibilities. From one point of view the increased viscosity of sperm-whale blood might call for changes in the circulatory apparatus over that of the humpback whale. From another point of view the decreased buffer property of the protein in the latter species would lower its power over the former in absorbing acids or bases during metabolism. It is conceivable that this point could have considerable significance in relation to special conditions tending to disturb the hydrogen ion concentration of the blood, the sperm-whale being considerably favored in this regard over the other species. In respect to human blood, at least, the protein of the blood of the sperm-whale is practically the same in quantity. As can be seen this does not hold true for the humpback.

The percentage of urea nitrogen in the non-protein content is much the same for both animals. In sperm-whale blood it is slightly over 60 per cent and in humpback whale blood 48 per cent. These are high values for mammals. They are also in excess of those for the blood of invertebrates, as well as for the marine and fresh water teleost and ganoid fishes, the blood of only the elasmobranch fishes exhibiting a greater percentage.

The quantity of the amino-acid nitrogen is also greater than that found in land mammals (1). In addition to this it makes up the larger part of non-protein nitrogen after subtracting the urea nitrogen.

The creatinine and creatine content is also relatively high for mammals.

The amount of uric acid found was not so excessive though its range more closely approximates that present in birds and reptiles.

The amount of the inorganic constituents does not differ greatly from that observed in human blood and other mammals. The effect of these substances on the osmotic pressure is very slight in comparison with that in the blood of the invertebrate animals.

The total solids and the specific gravity appear to vary considerably from human blood (specific gravity 1.026 to 1.030; solids 21,000 to 24,000 mg. per 100 cc.). The specific gravity of sperm-whale blood is much higher, though the solids are of a mean value.

The large amount of sugar in humpback whale blood appears to be rather excessive compared with human blood; that of the

sperm-whale, however, is much the same as that found in the latter. In both species it is much higher than that observed in the blood of invertebrates.

The cholesterol values obtained in the blood of both species are within the range found in human blood (30 to 60 mg. per 100 cc.), though very high compared to the amounts found in the blood of invertebrates.

The composition of whale blood seems to show several anomalies to that of other animals. Doubtless the peculiar habits of this animal and the fact that it is a marine mammal may help to account for these differences.

Further it is interesting to speculate on the special character of a metabolism which not only elaborates larger quantities of fat but the wax, spermaceti (cetyl palmitate), as well. Such phenomena only serve to make more impressive the marvelous lability of the chemical processes involved in the general metabolism of animal life.

SUMMARY.

Analyses of the blood of the humpback and sperm-whale were made. These analyses include the determination of specific gravity, solids, calcium as calcium oxide, chlorine as sodium chloride, total nitrogen, non-protein, urea, and ammonia nitrogen, creatinine and creatine, amino-acid nitrogen, uric acid, sugar, and cholesterol. These data are to be found in Table I.

The osmotic pressure of the blood of both species is undoubtedly higher than in other mammals ($\Delta = 0.7$–$0.9°$ approximately) but lower than that of the elasmobranch fishes and the invertebrates.

A considerable variation exists in the quantity of protein in the blood of the two species. In the sperm-whale it is nearly the same as that in human blood (21,000 mg. per 100 cc.). In the other species it is less than two-thirds as great.

The urea content is high for mammals, as well as the percentage of urea nitrogen in the non-protein fraction. Nor are the invertebrates or some fishes excepted in this respect, though the content of urea in the blood of the elasmobranchs exceeds that of whale blood.

The content of amino-acid nitrogen, of ammonia nitrogen, and of creatinine and creatine is generally higher in whale blood than in the other mammals, some marine invertebrates, and fish.

The uric acid content was not found to be excessive. It would probably range rather closely to that of birds and reptiles.

In respect to the amounts of the sugar and cholesterol, the former appeared to be excessive in the blood of only the humpback whale, though in the blood of the other species the amount found was similar to that found in human blood. The amounts of cholesterol in the blood of both species had much the same range of magnitude as is observed in human blood.

The amount of the mineral constituents was much the same as in other mammalian blood.

The specific gravity of sperm-whale blood is much higher than that of human blood, and for the other species it closely approximates the latter.

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